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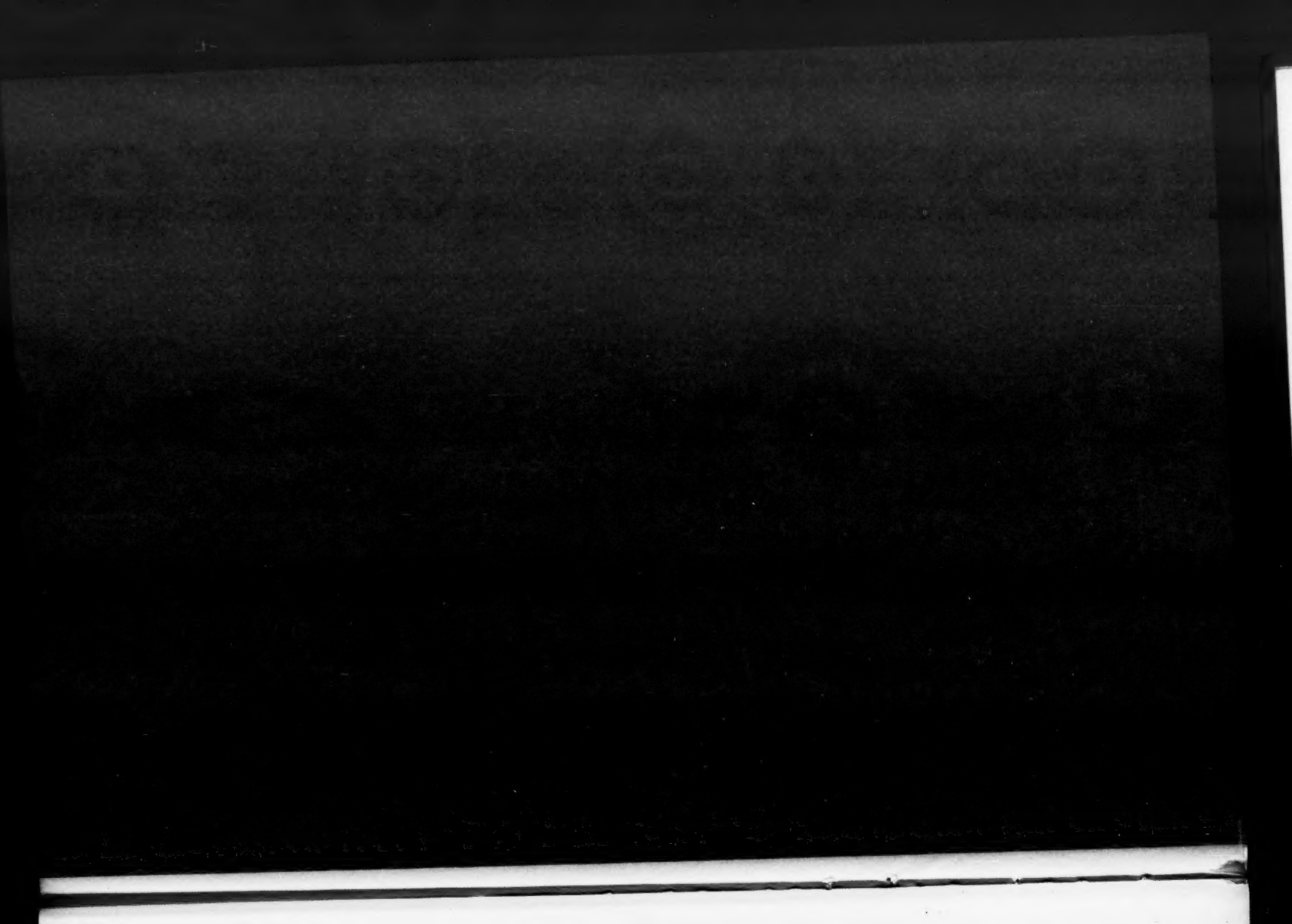

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MENDELSKA SÄLLSKAPET

LUND DEN 2 MARS 1953

THE COOPERATION OF GENOTYPES IN BARLEY

BY *ÅKE GUSTAFSSON*
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INTRODUCTION.

THE conception of natural selection, and its consequence the struggle for life, has in the course of time aroused the minds immensely. Here is not the place of analysing or reviewing all speculations, assumptions or conclusions. There is in my opinion no need to deny that the rude method of stamping-out, by which the »unfit» are eliminated from life, still forms a central theme of the evolutionary theory. It was vividly depictured by DARWIN himself. Let me cite from the »Origin of species» (Chapter III): »We behold the face of nature bright with gladness, we often see superabundance of food; we do not see, or we forget, that the birds which are idly singing round us mostly live on insects or seeds, and are thus constantly destroying life; or we forget how largely these songsters, or their eggs, or their nestlings, are destroyed by birds and beasts of prey; we do not always bear in mind that though food may be now superabundant, it is not so at all seasons of each recurring year».

In the following thesis not the method of stamping-out what is unfit will be considered, but the cooperation of fit and less fit to make the population, the entirety fitter than the partaking constituents themselves and alone. The mechanisms behind this cooperative increase in reproductive or vegetative fitness are barely touched upon in recent research. In many cases they are of a purely passive nature, making use, for instance, of all available space by means of differences in root and stem development, but now and then there may also occur some sort of a co-adaptation by biochemical means, an active attraction of biotypes or species to one another.

In this paper some examples will be given relating to an intraspecific type of cooperation. The theories and speculations involved in their interpretation will not be discussed.

An agricultural plant like barley forms an excellent material for the study of production capacity, of agricultural fitness, under varied

environmental conditions, in sparse or dense stands, with regard to competition within and between strains, or even with other species like oats. The production capacity can be exactly measured in vegetative properties, for instance tillering, or in generative characteristics like number of ovules and seeds, or kernel weight.

THE COOPERATION OF STRAINS.

The first part of this study deals with the important problem whether mixtures produce more than pure varieties. This is indeed a problem that has interested plant breeders and farmers for a long period of time. It was often considered that mixtures of strains and species, also within the self-fertilizing species, augment the yield per unit area (RÜMKER, 1892; MONTGOMERY, 1912; FRUWIRTH, 1923; ENGLEDOW and RAMIAH, 1930; ENGELKE, 1935; NUDING, 1936; JONES, 1948). This same doctrine has been essential to the Soviet-Russian ideas about competition within and between species [see, for instance, the results and compilations of CEDIK-TOMASEVIC, 1951: »Ausserdem geben die Mischungen von 2 Varietäten (einer Art) grössere Erträge, als gleichzeitig ausgelegte Komponenten im Reinanbau».] However, LYSSENKO, in his report of 1948, states as his opinion that neither competition, nor its contrast, here called cooperation, occurs within a species. (»Ablehnung des Kampfes und der gegenseitigen Hilfe der Individuen innerhalb einer Art.») There is no need to dwell on this unexpected controversy. It would be fatal, however, to forget about the empirical back-ground of CEDIK-TOMASEVIC's study. A negative attitude towards an improvement by means of blending characterized the conclusions of FRANKEL (1939), who in careful experiments analysed the yield properties of mixtures and pure strains. »In all nine trials the yields of the blends are very closely similar to the expectation ... Only in two out of the nine varieties is there any indication of an increased yield due to blending» (p. 254). CARDON (1921) did not notice any increase in yield after the mixing of wheat, oats and barley. His yield data are however rather irregular.

In order to study the problem very carefully the Golden, Maja and Bonus barleys were tested in 1951 with regard to their behaviour in mixtures and in purity. All three varieties are fairly related. Maja is a product of Golden and the Moravian Binder barley, Bonus a product of Maja × Seger × Opal, all of these derived from Golden crosses. The yield of the varieties runs as: Golden—100, Maja—115,2, Bonus—124,9 (FRÖIER, 1951). The figures refer to Svalöf conditions.

The seeds were sown with the help of marking-boards, in dense or in sparse stands, with two kernels or one kernel per hole of the board, with 200 or 500 kilograms of saltpetre per hectare. After the time of harvest the number of plants, spikes, kernels and kernel weights were determined for every plot, except in the case of two series (1 and 2), where two kernels per hole were sown. Here the number of plants could not be considered. The same set of experiments has been repeated and largely extended in 1952.

The series were: (1) 2 kernels per hole, 5 × 15 cm, 200 kg saltpetre, (2) like 1 but 500 kg, (3) 1 kernel, 5 × 15 cm, 200 kg, (4) like 3 but 500 kg, (5) 1 kernel, 10 × 15 cm, 200 kg, (6) like 5 but 500 kg, (7) 1 kernel, 10 × 30 cm, 200 kg., (8) like 7 but 500 kg. In this way it has been possible to trace directly the influence of density, manuring and mixture on seed production. Every plot consisted of plants from 90 sown kernels, except for plots 1 and 2, which were made from 180 sown kernels. There were consequently eight different plots of every strain and mixture, making in all 48 different plots.

The number of harvested plants is slightly superior for the pure strains, *viz.* 87,3 per cent of the sown seeds as against 86,2 per cent for the mixtures. The difference lacks significance.

The lumped data on spike and kernel number, kernel weights and 1000-kernel weight are as follows:

	Pure strains	Mixtures	Difference
No. of spikes	6998	7185	+ 2,7 %
» » kernels	164467	169057	+ 2,8 »
Total kernel weight.....	6688 gr	6936 gr	+ 3,7 »
1000-kernel weight	40,66	41,03	+ 0,9 »

In all examined properties the mixtures are somewhat superior, although the differences are not very striking. The higher number of spikes and kernels work together with the larger seed size to increase production by some four per cent.

Maja barley appears to be specially effective. The mixture of Maja and Golden lies 1,7 per cent higher than the best parent, Maja. Similarly, the mixture of Maja and Bonus surpasses the top strain, Bonus, by 2,4 per cent. The third mixture, Golden and Bonus, attains an intermediate position of the parents. In the experiments reported here the relative yields of Golden, Maja, and Bonus run as 100 : 110 : 116. The analysis implies that in this case two close-yielding varieties work better in mixture than do two distant-yielding types.

	Difference from best parent	Difference from mean of parents
Golden + Maja	+ 1,7 %	+ 6,4 %
Golden + Bonus	— 7,8 »	+ 0,1 »
Maja + Bonus	+ 2,4 »	+ 5,5 »

If the materials are analysed in more detail, distinct regularities can be traced (Table 1).

The mixtures (G + M, G + B, M + B) react in a similar manner. With dense sowings the mixtures are superior to the mean of the parents by 10 per cent, if the manuring is low. With high manurings the yield of the mixtures falls considerably (with circa 7 %). After sparse sowings and low manurings the mixtures are equal to the strains. With 500 kg manures they are again superior by 10 per cent.

The dense sowings comprise twelve plots of mixtures: six out of series 1 and 3, six out of series 2 and 4. The relative values of series 1 and 3 differ significantly from those of 2 and 4 ($P < 0{,}01$). Similarly the sparse sowings comprise twelve plots of mixtures. The six relative values of 5 and 7 differ from those of 6 and 8 ($P \geqq 0{,}05$). If we consider all 24 values, distributed into four groups as to density and manuring, there is a pronounced interaction of sowing density, manuring and strain mixture as to render a statistical value of $0{,}01 > P > 0{,}001$.

TABLE 1. *The relative effects of sowing distance and manuring on the production of pure strains in barley and their mixtures. Strains=100.*

	Dense sowings				*Sparse sowings*			
Seed distance	5×15 cm		5×15 cm		10×15 cm		10×30 cm	
Kernels/hole	2	2	1	1	1	1	1	1
Manuring, kg/ha	200	500	200	500	200	500	200	500
Series	1	2	3	4	5	6	7	8
All mixtures/strains	105,0	92,5	115,0	93,3	101,0	112,6	100,6	107,3

	Dense sowings		*Sparse sowings*	
Manuring, kg/ha	200	500	200	500
Series	1+3	2+4	5+7	6+8
G + M/strains	108,2	87,7	106,1	113,8
G + B/strains	103,7	94,4	92,5	108,8
M + B/strains	118,3	96,6	103,0	106,0
Average	110,1	92,9	100,5	109,5

(*G* = Golden, *M* = Maja, *B* = Bonus barley.)

Consequently, we may state that strain mixtures react in specific manners to the varied types of environment. It also follows that there is a distinct interreaction of genotypes. Under certain conditions the strains oppose, under other conditions they improve upon each other.

It is of fundamental value to know with certainty what lies behind the different production capacity of the mother strains. The average tillering of the strains amounts to 4,2, 4,1 and 4,3 spikes per plant, *i. e.* they lie on the same level. The increase in production rather depends on the higher number of kernels per spike and plant (84 → 89 → 96 kernels per plant) and the higher 1000-kernel weight (43,8 → 45,8 → 46,8 gr). These two properties work together in such a way as to raise the production of Maja barley and even more of Bonus barley to a very high level. The kernel weights per harvested plant is like 100 : 110 : 123 for the three strains, *i. e.* the same proportion for Golden and Bonus as in the Svalöf yield trials for a sequence of years (FRÖIER, *l. c.*).

The change in manuring from 200 to 500 kilograms per hectare results in a pronounced increase of tillering. For the lumped material it amounts to about forty per cent. This in its turn causes an increase in the number of kernels per plant. Since the 1000-kernel weight rises with the higher manuring, the increase in kernel number combined with the larger seed weight makes the total production considerably higher (37 and 47 per cent increase of weight per plant and plot, respectively).

The essential point of the study goes to show that mixtures may be superior to the pure strains, that this phenomenon often has a genetical back-ground, insofar as different strains react in a varying manner with one another — here Maja barley is the best combiner — and that the behaviour of the strain mixtures is directly influenced by the quality of the environmental factors (density, manuring).

In this series of experiments the factorial differences of the genotypes involved are no doubt very complex despite the close relationship of the strains. GUSTAFSSON *et al.* (1950) studied the behaviour of monofactorial mutants with regard to competitive ability. They showed that several mutations, homozygous or heterozygous, viable or lethal, interact with stand density, so as to be superior to the normal homozygote under certain conditions, inferior under others. In the analysis to follow a similar test material was studied with regard to the yielding capacity in mixture, *e. g.* segregating progenies of two monofactorial mutations.

YIELD INTERACTION IN MONO- AND DIHYBRIDS.

In a recent paper GUSTAFSSON (1952) studied the viability reactions of two induced mutations, alone and in combination. One was the erectoides mutation, ert_{29} (HAGBERG, NYBOM and GUSTAFSSON, 1952), characterized by a change in the spike density. It gives a certain decrease in viability, if adequately analysed. The other mutation, viridis, is a chlorophyll lethal, the recessive dying in the seedling stage. The two mutations are loosely linked, with circa 30 per cent crossing over. They arose in one and the same X_1 progeny.

In GUSTAFSSON's paper it was shown that ert_{29}, when heterozygous, decreased diploid viability. The chlorophyll lethal, vir, on the other hand, increased diploid viability when heterozygous. In the haploid state the chlorophyll lethal was eliminated to circa six per cent. ert_{29} showed no such gamete elimination. The result was that the two factors ert_{29} and vir behave entirely opposite to each other, both in the haploid and the diploid state.

In the year 1951 new experiments were begun and then fully analysed in 1952. The arrangements were as follows: In series 1—3 (*v.* below) seeds were sown of Maja barley in every tenth plot — 1, 11, 21, 31, *etc.* — each plot in series 1 and 2 comprising three rows and sixty seeds, in series 3 thirty seeds, in series 4 one hundred and twenty seeds, followed by randomized offspring of the normal homozygote (*AABB*), of the erectoides monohybrid (*AaBB*), of the lethal monohybrid (*AABb*), of the dihybrid (*AaBb*), of the erectoides homozygote (*aaBB*), and of the erectoides homozygote possessing the lethal (*aaBb*). In series 4 Maja barley was compared to the normal homozygote (*AABB*) and the erectoides mutant (*aaBB*).

Series 1: One kernel per hole of the marking-board, seed distance 5×15 cm, 300 kg saltpetre per hectare, 63 (64) offspring plots: Maja — 8, *AABB* — 6, *AaBB* — 7, *AABb* — 7, *AaBb* — 24, *aaBB* — 5, *aaBb* — 6. This arrangement corresponds to the so-called *standard environment* of GUSTAFSSON (*l. c.*), except for the fact that the manuring amounted to 300 instead of 200 kg.

Series 2: Like series 1 but 600 kg saltpetre, 51 (56) offspring plots: Maja — 7, *AABB* — 5, *AaBB* — 6, *AABb* — 3, *AaBb* — 21, *aaBB* — 5, *aaBb* — 4.

Series 3: Like 1 but seed distance 10×15 cm, 53 (55) offspring plots: Maja — 7, *AABB* — 2, *AaBB* — 6, *AABb* — 8, *AaBb* — 20, *aaBB* — 6, *aaBb* — 4.

Series 4: Two kernels per hole, 5 × 15 cm, 300 kg saltpetre. 12 (15) plots: Maja — 2, *AABB* — 5, *aaBB* — 5.

Comparison of Maja barley and the normal homozygote (AABB). — This analysis was made in order to settle whether the original irradiation had in any way influenced viability of the normal homozygote extracted from the segregating material of the X_2 generation. Let me once and for all conclude that the normal segregate of the dihybrid (*AaBb* → *AABB*) is equal to the mother strain in production. It was tested not merely in the series and plots described above but also in a few yield trials of the Barley Department of the Swedish Seed Association. The following illustrates the case:

	1951, series 1—4			1950—1951, yield trials		
	Yield/plot, grams	Rel. prop.	No. of plots	Yield/plot, kg.	Rel. prop.	No. of plots
Maja barley	229,3	97,7	24	6,56	104,1	3
Normal homozygote	235,4	*100*	18	6,30	*100*	3

Grand total: Maja barley = 100,9
Normal homozygote = *100*

In the following discussions for this reason, when dealing with plot production, the values with regard to Maja barley have been added to those of the normal homozygote.

GENOTYPE VIABILITY.

The erectoides mutation. — In the summer of 1951 all material was classified with regard to the mode of segregation, whether it contained recessives of the erectoides or viridis factor, or both of them. Offspring producing viridis mutants were then tested indoors. In the summer of 1952 a great many progenies were further analysed with regard to the occurrence of normal homozygotes and monoheterozygotes of ert_{29}.

The 1951 plots segregating for the erectoides mutation and fully analysed in 1952 gave the following results with regard to genotype viability, in this case kernel weight/plant (normal homozygote = 100):

	AABB	*AaBB*	*aaBB*	Average plant yield	No. of plants
Series 1	100	90,6 ± 6,3	81,3 ± 6,7	4,42 gr	180
» 2	100	102,2 ± 14,0	80,7 ± 9,1	4,67 »	56
» 3	100	98,8 ± 9,1	96,9 ± 9,9	7,18 »	168
Average	100	97,2	86,3	—	404

The results of series 1 (the standard test) entirely agree with the data given by GUSTAFSSON (*l. c.*, p. 272). Here the proportion runs as 100 : 91 : 81, there as 100 : 94 : 79. Consequently, in standard sowings with 200—300 kg saltpetre the erectoides mutant is inferior to the mother strain by 20 per cent. The monoheterozygote is intermediate in production. With a higher manuring (series 2) the heterozygote is apparently more productive, but no statistical significance of the fact is found. With a less degree of competition (series 3) there is an increase in relative production of the mutant. The heterozygote is still intermediary. The mutant is definitely better than in the dense sowings.

In the offspring of dihybrid plants no three but nine different genotypes are competing, three of which (*AAbb*, *Aabb*, *aabb*) die at an early stage. Six consequently survive. Without any detailed analysis it may be stated that the production of *AABB*, *AaBB* and *aaBB* is in the proportion of 100 : 98 : 78, *i. e.* figures similar to those given above.

Other facts than those reported add up to the following conclusion: Erectoides 29 is in the case of a high stand density definitely inferior to the competing genotypes. In the case of a more open growth with less competition (sparse stands) it may approach the normal homozygote. This holds true of pure cultures as well, either in small plots or in normal yield trials. 21 small plots gave an average relative production of 92,1 %, 5 plots from yield trials the relative production of 93,6 %.

The viridis mutation. — In the following survey all the 1951 data on the viridis heterozygote have been gathered. They concern monohybrid offspring exclusively — *AABb* → *AABB*, *AABb* and *AAbb* —, where the *AAbb* genotype is lethal.

	AABB	*AABb*	Average plant yield	No. of plants
Series 1	100	102,7 ± 6,8	5,29 gr	314
» 2	100	110,8 ± 9,5	6,07 »	122
» 3	100	112,3 ± 6,6	8,11 »	182
Average	100	108,6	—	618

Evidently the lethal heterozygote is by 8 or 9 per cent superior to its mother strain. In the material studied by GUSTAFSSON (1952) it was to circa 20 per cent superior. Nevertheless the new material confirms the chief results of the previous study.

It is interesting to note that the viridis heterozygote is more productive in the lack of competition (series 3) than in the corresponding

crowded conditions of series 1. This contrasts to the spontaneous lethals albina 7 and xantha 3 arisen ouf of Golden barley (GUSTAFSSON, NYBOM and VON WETTSTEIN, 1950), which are superior under crowded conditions but inferior when competition relaxes.

Erectoides and the viridis mutation. — In the 1949 experiments (GUSTAFSSON, *l. c.*, p. 271) the data taken at their face value indicated that the genotype, homozygous for ert_{29} but heterozygous for vir (*aaBb*), was inferior to its sister genotype *aaBB*, homozygous for ert_{29} and normal chlorophyll formation. However, the difference was not significant ($P = 0{,}1$). In the new experiments this point was specially studied, with the following result.

	aaBB	*aaBb*	Average yield/plant	No. of plants
Series 1	100	107,7 ± 6,5	4,73 gr	267
» 2	100	97,3 ± 7,2	5,34 »	150
» 3	100	109,5 ± 12,1	7,12 »	81
Average	100	104,8	—	498

The analysis gives no clear-cut answer to the problem. The standard errors are high and the differences fairly slight. The material certainly indicates a weak superiority for the viridis lethal also with the erectoides genotype. Like the case with the lethal in the normal genotype its greatest effect is when the crowding is relaxed (series 3). On the other hand, if we average all the data from 1949 and 1951, we obtain the following relative values: 88,5, 107,7, 97,3, 109,5, with a mean of 100,8, *i. e.* almost the exact production of the homozygous erectoides genotype (*aaBB*). The erectoides genotype shows a wide variation in production. This is even more so for the lethal heterozygote, in changed environments and different years.

The offspring of dihybrid plants contain three different genotypes, possessing heterozygous lethals, *viz. AABb*, *AaBb* and *aaBb*. The values of relative production are as 98 : 104 : 82 (*AABB*=100). The erectoides homozygote *aaBB* is to 1,3 per cent inferior to its lethal heterozygote. Consequently here, too, the lethal heterozygote appears to exert a slightly beneficial effect.

The genotype viability in dihybrid offspring. — Owing to the low number of normal homozygotes appearing, *viz.* one normal segregate out of twelve surviving individuals in the case of free combination and less in the case of factor repulsion, there is a great labour involved when trying to obtain sufficient material. The interpretation of the data is not easy. They are given with all reserve.

	AABB	*AaBB*	*AABb*	*AaBb*	*aaBB*	*aaBb*	Yield/ plant	No. of plants
Series 1	100	102,3	104,7	*106,0*	78,4	81,4	4,97	219
» 2	100	94,2	100,0	*103,1*	81,7	92,0	5,25	175
» 3	100	96,3	89,1	*102,8*	82,3	72,9	8,10	165
Average	100	97,6	97,9	*104,0*	80,8	82,1	—	
No. of plants	55	86	72	186	72	88	—	559

Except for the production of the erectoides genotype *aaBB*, the figures scarcely agree with those given by GUSTAFSSON (1952, p. 272). In the old analysis, however, the kernel yields were not examined, merely the number of spikes and kernels. In addition, there were only six normal plants present, giving a high statistical uncertainty. Especially interesting in the new analysis is the high production of the dihybrid itself, which is 4 per cent better, on the average, than the normals. In all three series it lies higher than any other genotype. Still the proportions are statistically uncertain.

Summarizing the data given, we conclude with regard to the production capacity of individual genotypes, either in pure cultures, in monohybrid or in dihybrid segregation: (1) the erectoides homozygote is by 20 per cent inferior in segregating progenies of normal density, but no more than 5—10 per cent in pure cultures, (2) the erectoides heterozygote *AaBB* lies closer to the normal homozygote, but is still by at least 3 or 4 per cent inferior, (3) the erectoides type heterozygous for the lethal, *aaBb*, is similar to the erectoides homozygote *aaBB*, possibly somewhat superior, (4) the normal type heterozygous for the lethal, *AABb*, is distinctly superior to its homozygote *AABB* by 10 per cent or more, and (5) the double heterozygote *AaBb* appears to be slightly superior to all other genotypes in the case of a dihybrid segregation.

THE REACTION OF GENOTYPE MIXTURES.

The erectoides monohybrid. — In Table 2 the data do not refer to the production of individual genotypes but of genotype mixtures, the mother plants of the plots having the constitution *AABB*, *AaBB*, *aaBB*.

Most interesting figures relate to the offspring of the erectoides monohybrid *AaBB*, with its three segregated genotypes of *AABB*, *AaBB* and *aaBB*. The yield data of this offspring lie in all three series above the calculated mean of the individual genotype productions, but also, on an average, above the normal homozygote with no less than four

TABLE 2. *Production of the monohybrid offspring in relation to its corresponding homozygotes.*

	AABB-offspring, per plot (gr)	*AaBB*-offspring (rel. fig.)	*aaBB*-offspring (rel. fig.)	Per cent of harvested plants *AABB*	*AaBB*	*aaBB*
Series 1	233,0	107,2	98,7	86,3	94,3	88,3
» 2	262,8	99,4	83,6	90,4	93,1	83,0
» 3	199,3	105,5	94,1	93,0	98,9	92,8
Average	100	104,0	92,1	89,9	95,4	88,0

per cent. If we apply the yield figures found for the three genotypes within a segregating progeny, we should expect the *AaBB* offspring of series 1 to produce 90,8 per cent of what *AABB* is producing, of series 2 to produce 97,0 per cent and of series 3 to produce 98,5 per cent, *i. e.* an averaged yield of 95,4 per cent of *AABB*. Since we know the production of the individual offspring plants of the normal homozygote in pure culture, we can compare these values with those actually found within the segregating progenies.

Individual plant production in pure cultures and genotype mixtures. (Relative figures.)

	Pure cultures		Genotype mixtures All plots	
	AABB	*aaBB*	*AABB*+*AaBB*	*aaBB*
Series 1	100 (4,50 gr)	96,4	100,0	91,3
» 2	107,6	98,0	107,3	91,3
» 3	158,9	150,0	163,1	150,7

The higher production of the monohybrid offspring depends on two factors: (1) a higher number of harvested plants per plot, 95 % as against 90 % of the seeds sown, (2) on a higher production capacity of the individual plants in genotype mixtures. If we scrutinize the figures above, we find that the genotypes *AABB* + *AaBB* lie on the same level as or higher than the corresponding normal plants from the pure cultures. Taking into consideration that *AaBB* is inferior to *AABB* by some three per cent, we may conclude that also *AABB* itself produces more in genotype mixtures than in pure cultures.

It is, however, not quite appropriate to lump all yield values and to compare the lumped figures for genotype after genotype, since the percentage of harvested plants is so different. If we for that sake divide the materials into groups of different density, according to the number of harvested plants per plot, we obtain the values of Table 3.

TABLE 3. *Production of the erectoides monohybrid in relation to the two homozygotes and the occurring plant density. (Calculated as harvested plants/sown seeds.)*

	Series 1		Series 2		Series 3	
	80—90 %	90—100 %	80—90 %	90—100 %	80—90 %	90—100 %
AABB-offspring ..	239,1 gr	221,9 gr	235,2 gr	268,3 gr	220,5 gr	196,5 gr
No. of plots	9	5	2	10	1	8
AaBB-offspring ...	247,8	250,8	255,0	264,1	—	213,0
No. of plots	2	5	2	4	—	6
aaBB-offspring ...	[190,8]	256,0	203,3	285,5	148,0	195,5
No. of plots	2	3	4	1	1	5

Series 1—3. Relative values:		80—90 %	90—100 %
	AABB	100	100
	AaBB	106,0	106,6
	aaBB	77,8	107,1

The results are immediately evident: (1) The erectoides progeny (*aaBB*) is much better when a full number of plants is present. In fact, it is by some 7 per cent better than the normal type in the case of full density but is inferior in sparse stands by some 20 per cent. The difference appears to be significant ($0{,}05 > P > 0{,}01$). This indicates that the erectoides genotype is a good yielder in the case of a dense stand. When for some reason, however, there occur gaps in the stand or the germination is depressed, it shows a poor compensation ability by means of an increased tillering. This is in agreement with other experiments and explains why erectoides cannot compete in mixed stands but is better in pure cultures, relatively seen.

(2) The monohybrid mixture (the *AaBB* offspring) is better yielding than the normal type both in dense and sparse stands. It is, on an average, superior by 6 or 7 per cent in both density groups. Also here the significance appears to be reliable ($0{,}05 > P > 0{,}01$). Consequently, the monohybrid mixture owes its superiority not only to the higher plant percentage, but to a better yielding per plant, since it is superior both when the plant number is maximal and when it is decreased.

The lethal monohybrid. — The data are summarized in the following survey:

	Yield of *AABB*-offspring per plot	Yield of *AABb*-offspring (relative figures)	Per cent harvested plants, *AABB*	Per cent harvested plants, *AABb*
Series 1	233,0 gr	102,0	86,3 %	74,4 %
» 2	262,8 »	94,3	90,4 »	68,3 »
» 3	199,3 »	92,5	92,9 »	74,3 »
Average	100	96,3	89,3 %	72,4 %

In spite of the much decreased number of plants per plot the lethal heterozygote is entirely on the level with the normal type in series 1, the standard arrangement. However, it is distinctly inferior in the case of a sparse stand (series 3). In series 2, where the number of harvested plants is extremely low, it also falls down. Nevertheless its yield capacity is astonishingly good. Apparently it has a high compensating ability, fills out the gaps arisen from the death of the chlorophyll recessives, when the gaps are not too pronounced as in the case of 2 and 3.

This ability of compensation is more pronounced in the genotype *AABb*, the lethal heterozygote, than in *AABB*.

Average plant production. (Relative figures.)

	Homozygote offspring	Heterozygote offspring	
Series 1	100 (4,50 gr)	117,7	*AABB*: 115,7 *AABb*: 118,8
» 2	107,6	134,3	*AABB*: 127,2 *AABb*: 140,1
» 3	158,9	180,2	*AABB*: 167,6 *AABb*: 188,3

It is plain that a high manuring (series 2) causes a rise in average plant production, although smaller than with a wide seed sowing. In all three kinds of environment the heterozygote compensates better than the normal type to an increase in available plant space.

What has here been concluded, is confirmed by a detail analysis of the different density groups. There is in this instance, too, especially in series 1 but also noticeable in series 2 and 3, an evident occurrence of a genotypical cooperation.

Erectoides and the lethal heterozygote. — In this case there is but a weak compensation for the decrease in plant number, even in the

	Yield of *aaBB*-offspring per plot	Yield of *aaBb*-offspring (rel. fig.)	% of harvested plants, *aaBB*	% of harvested plants, *aaBb*
Series 1	229,9 gr	92,1	88,3	75,3
» 2	219,8 »	91,2	83,0	62,5
» 3	187,6 »	76,8	92,8	67,5
Average	100	86,7	87,3	68,4

standard arrangement of series 1 and with the high manuring of series 2. The decrease in yield is very pronounced, when the wide sowing of series 3 is considered.

The two erectoides genotypes do not possess the same compensating ability as the corresponding normal genotypes. In addition, the *aaBb* plants are less superior to the erectoides homozygote *aaBB*. There is however a small degree of genotype cooperation apparent when the different percentage groups of harvested plants per plot are compared for the two types of offspring.

Finally, it is impossible at present to discuss the results of the *dihybrid offspring* plots. So much can be said, however, that the individual genotypes have, on an average, a lower compensation ability than the normal lethal heterozygote but a higher than the erectoides lethal heterozygote, for the corresponding percentage groups of harvested plants.

CONCLUSIONS.

In a series of papers the present author and his collaborators have tried to exemplify the complex modes of interaction between genotypes, as well as genotypes and environment, also when monofactorial differences are studied. A specially significant phenomenon deals with the so-called Montgomery effect. This implies that a special genotype, although inferior in maximal production capacity, nevertheless might be superior in competition. This feature was studied by GUSTAFSSON *et al.*, 1950, in some monofactorial mutations in barley, viable as well as lethal. Another significant result of recent research has indicated that a mutant may react in a most unexpected way to a drastic change in environment. Here the ancestor line becomes scarcely viable and is often surpassed in viability by a normally inferior or even semilethal mutant. In addition, mutations lethal or semilethal, when homozygous, may stimulate in heterozygous condition and make the heterozygote superior in production to its normal homozygote.

In this paper some further contributions have been rendered. Take, for instance, the lethal viridis mutation, induced in Maja barley by X-rays. It causes one fourth of the monohybrid offspring to die at an early seedling stage. In spite of the necessarily thinned stand the heterozygous lethal conditions such a high compensation ability of the monoheterozygote and, by genotype cooperation, of the normal homozygote, too, as to make the population reach or surpass the production level of what the normal homozygote does in pure cultures. Here it is

worth while to refer to the publication of ENGLEDOW and RAMIAH (1930), who found that 20 to 30 per cent of the possible maximal yield of a cereal field is regularly lost by gaps and thinnings in the population. Some ten years later TEDIN and ANDERSSON (1943) noted that neighbours of the existing gaps in a barley field were able to compensate for approximately one third of the total loss in production, caused by plant accidents. The remaining two thirds of the loss could not be regained by an increase in tillering. The proportion of $^1/_3$ regain, $^2/_3$ loss, of course, depends on the choice of genotype, the type of climate and the actual environmental conditions. The viridis heterozygote of Maja barley, discussed here, and by compensation and genotype cooperation, its normal homozygote, too, together recapture more than 100 per cent of the loss. This is when the sowing arrangements are of the standard type, *i. e.* at a normal density. The seed distance being exceptionally large or the plant elimination heavily accentuated, the offspring of the lethal heterozygote are incapable of compensating the entire loss.

Even more pronounced is the cooperation when the offspring of the erectoides monohybrid are considered. Here the mixture of $^1/_4$ *AABB*, $^2/_4$ *AaBB* and $^1/_4$ *aaBB* produces four per cent more than the normal homozygote itself, despite the fact that *AaBB* is by three per cent inferior and *aaBB* by fourteen per cent inferior to the *AABB* genotype of the same offspring. Under the standard arrangement the surplus lies higher, *viz.* between seven and eight per cent. In his paper of 1912, MONTGOMERY stated that two varieties in competition gave »a greater number of plants at harvest». This is valid here, too. (The strain mixtures, however, gave a lower plant percentage than the pure strains. *Cf.* p. 3.) In addition, there is a distinct gain in plant productivity, compared to the conditions in pure cultures.

Further points of interest concern the behaviour of the lethal and the erectoides heterozygotes. It has again been found that the genotype heterozygous for the lethal, *AABb*, increases production above that of its homozygote, but that the erectoides heterozygote, *AaBB*, on the other hand, decreases production. This illustrates once more »that there are no definite means of calculating from the viability of the homozygous mutation, how it will react in the heterozygous state» (GUSTAFSSON, 1952, p. 272). Interesting, too, is the fact that the induced lethal is equally effective in sparse as well as in dense plant stands. This is in contrast to the spontaneous lethals previously studied in this respect (GUSTAFSSON, NYBOM and VON WETTSTEIN, 1950), which

are superior under competition but inferior when competition relaxes. The reason, why the viridis lethal increases yield, is so far not settled. There is, however, a weak indication that the superiority is connected with an increase in chlorophyll pigments (NYBOM, unpubl.), similar to what was found in the chlorophyll heterozygote of *Antirrhinum* studied by STUBBE and PIRSCHLE (1941).

The behaviour of the ert_{29} homozygote further illustrates the complexity of the viability reaction. Under heavy competition (dense stands) the mutant is by 20 per cent inferior to its ancestor. When competition is relaxed, but still in segregating progenies, it gains in relative production up to a 4 per cent point of inferiority. Similar is the case in pure stands, either in the small plots of series 1—4 or in regular yield trials, where its inferiority stops at 5—10 per cent. This contrasts to the bright-green mutant 4 out of Bonus barley (GUSTAFSSON and NYBOM, 1950) that is superior to its ancestor in dense stands but distinctly inferior in yield trials or in such mixed stands where competition relaxes.

These data further argue the need of extreme caution when evolutionary trends are concluded out of the behaviour of some single mutation studied. In barley every mutation, so far studied, whether morphological or physiological, drastic or minor, viable or lethal, appears to possess a reaction mode of its own, changing with the type of environment and the genotypical milieu.

There is consequently a wide complexity of genotype interreactions. The stamping-out method of natural selection, so expressively depicted by DARWIN, has a counter-part in the mutual cooperation of genotypes, within or between species. If the results of this paper are valid not only for the agricultural field, but can be transferred also to the happenings in free nature, one important conclusion turns up. Although a certain mutant, variety or species, owing to an inferiority in production, should according to advance calculations be doomed to extinction, it will be tolerated or even favoured as a member of a genotype community, since it enhances fitness of its superiors and of the population as a whole. This is specially illustrated by the monohybrid offspring of ert_{29}, *AaBB*, as well as the Golden-Maja and Maja-Bonus mixtures. A population, lacking in inferior genotypes and in heterogeneity, should according to this view often be less fit in total production than a corresponding population hiding harmful genes or producing inferior genotypes.

To finish this sketchy analysis: There is successively spreading in

agricultural praxis a belief that a superior cereal stock should not be built upon a single genotype, but rather on a couple of or on numerous genotypes, selected so as to cooperate, also in self-fertilizing organisms. Such a view obtains some support from this study. The plant breeder making hybrid corn or sugar beet selects the inbred lines or strains which are specially apt in intercrossings. In a similar way the wheat, oats and barley breeders have in future to select the lines or strains that interreact in an improving way upon the production of the population and by that lead forward to a gain in total yield.

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INBREEDING IN AUTOTETRAPLOID RYE

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DATA from inbreeding investigations in autotetraploid rye have been published by LUNDQVIST (1947). In a limited material it was demonstrated that the tetraploids showed a less pronounced inbreeding depression in the I_1 than did the corresponding diploid variety. Since that time, data have been collected from continued inbreeding up to the sixth generation. As this material may be of interest in elucidating some aspects of inbreeding phenomena, it has been considered worth publishing in part at this time.

MATERIALS AND METHODS.

The material has been obtained by selfings, starting with élite diploid and tetraploid populations of Steel-rye. Each year a new I_1 population was obtained from the élite population of that time. At the same time, existing inbred populations were propagated by selfing. Therefore the different inbred populations growing side by side in the field each year were not descendants from one another, nor were they from the same original parental population. In order to present as representative a material as possible within a limited space, it has been considered more convenient to take a cross-section from the material of a certain year (*e. g.*, the first four unrelated inbred generations grown in 1950), rather than taking a longitudinal section by following the progress of continued inbreeding in a number of inbred lines, as the seasonal variations may cause quite considerable fluctuations. In order to overcome the difficulties imposed by the variation in self-fertility of the material and to make the different inbred generations as comparable as possible, the self-sterile lines will not be treated in this paper, and in the self-fertile material each line will be represented by one family only, this being the total number of families in many lines. Because of the variation in numbers of seeds sown, in sprouting ability, and in seedling lethality, the presented lines, nevertheless, are characterized by rather differing numbers of individuals. The distribution of the material is shown in Table 1 *a*.

TABLE 1. *Distribution of the inbred material.*

		Total number of individuals	Number of I-lines	Number of families	Number of individuals pro family	Average number of individuals
		a. *The population inbred material.*				
4*n*	I_1	199	22	22	5—16	9
	I_2	214	22	22	5—19	10
	I_3	169	22	22	5—10	8
	I_4	144	17	17	6—10	8
	Pop.	748	—	—	—	—
2*n*	I_1	173	14	14	4—32	12
	I_2	174	22	22	3—10	8
	I_3	169	22	22	4—10	8
	I_4	166	21	21	3—10	8
	Pop.	734	—	—	—	—
		b. *The chimaera inbred material.*				
4*n*	I_1	30	3	4	4— 9	8
	I_2	92	3	12	2—10	8
	Pop.	109	—	—	—	—
2*n*	I_1	43	3	6	4—10	7
	I_2	92	3	15	1— 9	6
	Pop.	82	—	—	—	—

It is important at this point to consider the three following questions: (a) To what extent can the élite populations of different years be considered to be genetically alike? (b) Does the number of plants producing inbred progeny from the different élite populations constitute a representative sample of different genetic factors and degrees of heterogeneity in this material? (c) Does the number of raw tetraploids, once obtained from the diploid strain, form a representative sample of the factors found in that strain?

As to question (a), LJUNG (1948) has outlined the program of mass selection which is practiced at the Seed Association in Svalöf in order to maintain the level of commercial varieties of rye. The effective size of the population seems to be rather small, thereby permitting considerable genetic drift. However, the rigorous selection indicated in this program appears to counteract this drift in large part. The influence of seasonal variations on this selection is difficult to evaluate. The degree of genetic similarity of élite populations from different years must evidently be judged with caution. This applies especially to the young tetraploid strain which scarcely can have reached a geno-

typic equilibrium, adapted to the selection applied, that characterizes long-derived highbred populations such as the diploid strain.

Questions (b) and (c) are quite similar. The samples of selfed plants from the different élite populations comprised 14—22 plants. According to MÜNTZING (1951), the tetraploid strain of Steel-rye originated from seven colchicine-treated diploid kernels (the number of 9 kernels given on p. 18, *l. c.*, is not correct; personal communication from the author). The following calculations are based on the diploid condition and afford some elucidation of the questions (b) and (c).

The expression $[(p^2)^n + (q^2)^n]$, derived from the expansion of $(p^2+2pq+q^2)^n$, expresses directly the probability of obtaining only one of the alleles of a biallelic segregating locus in any sample of n individuals in which p and q represent the proportions of the two alleles, and $p + q = 1$. When a single locus is considered, with $p = q = 0{,}5$, the probability of obtaining both alleles in a sample of 7 individuals is 16382/16384 or $0{,}_{99987}$. When 500 segregating biallelic loci are considered, the probability of obtaining all alleles in a sample of 7 plants is $0{,}_{942}$. When $n = 22$ and $p = q = 0{,}5$, one is practically certain to obtain all of the segregating alleles, even for a very large number of segregating loci.

These probabilities are considerably diminished when p and q differ. Assuming $p = 0{,}9$, $q = 0{,}1$ and $n = 7$, the probability of obtaining all the alleles of 500 segregating loci is exceedingly small, as can be seen from the corresponding low probability of $0{,}_{00748}$, *i. e.*, less than 1 %, for a sample of 22 individuals. However, such variations in allelic proportions are accompanied by the diminished importance of the less frequent allele. Therefore, it may be justified to consider the bulk of genes of the élite populations to be rather well represented in a sample of 20 individuals from a qualitative point of view. This holds true, even to a greater degree, for the tetraploids, as can easily be realized from their segregation behaviour. On the other hand, the 7 plants which formed the basis for the tetraploid strain of Steel-rye probably constituted an insufficient sample of the bulk of genes in the original diploid population.

The quantitative aspects of the representation of genes in the samples in question can be examined by developing the expression $(p + q)^{2n}$. In the different binomial series obtained, $p =$ mean and $\sqrt{\frac{pq}{2n}} =$ the standard error. The following table will give some idea of the representativity of the samples.

		Standard error when		
$p:q$	$\bar{x}$	$n=7$	$n=22$	$n=50$
0,5 : 0,5	0,5	0,13379	0,07537	0,05000
0,75 : 0,25	0,75	0,11576	0,06527	0,04336

It can be seen from the table that the quantitative conditions are rather unsatisfactorily reflected in a sample of 7 individuals. A sample of 22 individuals would, on the other hand, give a rather satisfactory statistical dispersion. In the preceding calculations, the presence of multiple alleles has not been considered. From that point of view a sample of 22 individuals may be too small, while a sample of 50 individuals would appear to be on the safe side both from a qualitative and a quantitative standpoint.

With regard to the degree to which the samples adequately represent the varying heterogeneity of élite populations, it is evident that they will consist primarily of plants with intermediate numbers of segregating loci. The degree of uniformity in sample heterogeneity will be determined by the number of segregating loci in the population and the number of individuals in the samples. The standard errors of the sample means can be expressed as $\sqrt{\frac{hpq}{n}}$ where $p+q=1$ and are the symbols for the average proportions of homozygous and heterozygous allelic combinations, respectively, in segregating loci; where $h=$ the number of segregating loci; and where n means the number of individuals in the sample (BONNIER and TEDIN, 1940). Considering the probably very high value for h, it seems justified to assume that even with some variation for segregating loci between populations from different years, the samples can be regarded as fairly comparable with one another.

It is evident from these considerations that the most important source of error in this comparative study of inbreeding effects in diploid and tetraploid populations is that of the doubtful representativity of the tetraploids as to the bulk of genes of their diploid origin. In order to overcome this source of error, the experiments were extended to a material obtained from diploid-tetraploid sectorial chimaeras. Three such plants, derived from open-pollinated seed on a single maternal plant, were produced by colchicine treatments. The three plants were self-fertile in all parts. Data from I_1 and the corresponding

I_2 generations are summarized in Table 1 *b*. It must be observed that in I_1 clonal lines have been put equivalent to families.

All plants were grown in the field. Seeds were sown with the use of marking boards, and were spaced at 10 cm. intervals in rows separated 15 cm. As a standard material every third row consisted of common élite population. Owing to the paucity of seeds in many cases, it was not possible to replicate the trials. When selfing, it has not been possible to choose plants quite at random as it has been necessary to avoid the weakest plants in order to get sufficient seed to perpetuate the inbred lines.

Data on several characters were collected from the material. Only the data collected on the following three characters will be considered here: (a) plant height, (b) number of spikelets in the largest inflorescence, and (c) open-pollinated seed sets. These characters are relatively simple and uncomplicated, thereby facilitating an anlysis of the effects of inbreeding. At the same time they represent the vegetative, the generative, and to a certain extent an intermediate phase in plant development.

EXPERIMENTAL RESULTS.

In order to facilitate direct comparisons between different materials and different characters, the absolute values of the means in different characters have for each family been transformed to per cents of the mean of the two adjacent rows of standard material. The transformed values for the three characters studied are tabulated in Table 2, which includes material derived from the élite population and from the sectorial chimaeras. In order to show the variation between families in the chimaera-derived material, these means have been included separately in the table. However, the lines in this material were not represented by an equal number of families, and generation means were therefore calculated on the means of lines.

Attention should be drawn to the fact that the values obtained for seed fertility in the chimaera material are not representative, as they do not include all plants from which other data were available. Many plants tillered poorly and did not have enough ears for both self- and open-pollinated seed sets. After selfing a number of plants have been left, and these have mostly been the weak ones, not suitable for isolation, thus representing the result of a negative selection.

The progress of the inbreeding depression of the diploid and the tetraploid material in various characters is contrasted in Fig. 1, where

TABLE 2. *Means of I-families in per cent of population means.*

		15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	105	110	115	120	n	$\bar{x}$	m	Diff.	m_{diff}	t
Plant height in cms	1. 4*n* I_1																7	2	8	3	2			22	95,45	1,4211	1— 7= 7,95	2,5855	3,07**
	2. I_2														1	6	4	7	3	1				22	89,32	1,3782	2— 8= 1,37	3,2139	0,43
	3. I_3											1	1	1	2	1	8	3	2	3				22	87,27	2,2905	3— 9= 7,95	2,8687	2,77**
	4. I_4													1	1	2	7	3	2	—	1			17	88,68	1,9426	4—10= 4,51	2,9076	1,55
	5. Ch I_1											1	—	3										4	71,01				
	6. I_2											3	1	4	3	1								12	69,71				
	7. 2*n* I_1													1	1	3	5	1	2	1				14	87,50	2,1611			
	8. I_2										1	—	—	2	2	4	5	4	—	1	1	1	1	22	87,98	2,9037			
	9. I_3												3	3	6	7	1	—	2					22	79,32	1,7266			
	10. I_4												3	2	1	4	3	6	2					21	84,17	2,1639			
	11. Ch I_1													2	—	1	3							6	81,92				
	12. I_2									1	—	—	4	2	1	4	2	1						15	76,22				
spikelets	13. 4*n* I_1														1	—	1	7	3	4	3	3		22	98,86	1,9233	13—19= 9,93	3,7759	2,63*(*)
	14. I_2											1	—	—	1	7	6	6	1					22	86,14	1,5494	14—20= 8,41	2,3413	3,59***
	15. I_3								1	—	1	—	—	3	2	4	5	4	—	2				22	82,73	2,7617	15—21=14,55	3,9172	3,71***
	16. I_4											1	1	2	4	6	3							17	78,97	1,7023	16—22=12,42	3,3449	3,71***
	17. Ch I_1										1	1	—	1	1									4	68,97				
	18. I_2									1	2	4	1	1	2	1								12	65,85				
	19. 2*n* I_1																2	2	—	5	2	—	1	14	88,93	3,2492			
	20. I_2																2	1	5	5	6	2	—	22	77,73	1,7556			

| Number of Seed fertility in per cent | | |

the curves for the population descendants and the chimaera descendants have been based on the common means for the various inbred generations.

It can be seen from the curves in Fig. 1 that the reaction of diploids and tetraploids to inbreeding is similar in different characters. Plant height is least affected, while seed fertility is most affected. However, the values for the tetraploids are in all characters and gene-

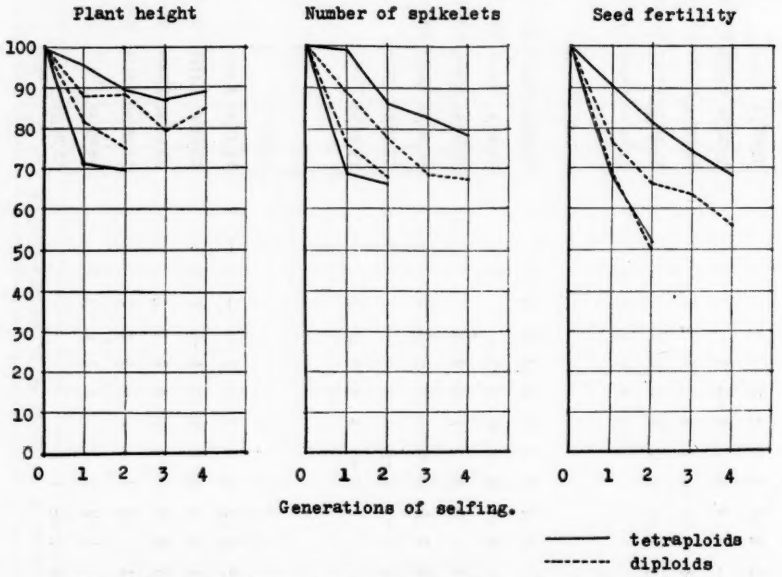

Fig. 1. Curves for depression by selfing. (Explanation in the text.)

rations beyond those of the diploids, and the »*t*» values for these differences (the quotients of differences and corresponding standard deviations) are often highly significant. The statistical significance of these values has been indicated in accordance with the methods of BONNIER and TEDIN (1940). The chimaera-derived populations were too limited to permit more detailed analysis. Nevertheless, the same tendencies of inbreeding sensitivity in different characters are also evident in this material. The more plain slope of depression is even more pronounced here. The original chimaeric tetraploids have probably had a vitality at least comparable to the lower part of the variation range for I_3 and I_4, obtained from a population élite. It should therefore be remembered

TABLE 3. *Correlation coefficients for the population inbred material.*

	Tetraploids				Diploids			
	I_1	I_2	I_3	I_4	I_1	I_2	I_3	I_4
Height—spikelets	0,4202(*)	0,6601***	0,6823***	0,5625*	0,3774	0,4375	0,9476	0,3101
Height—seed fertility	0,4628*	0,5947**	0,5941**	—0,2300	0,5977*	0,6566***	0,3244	0,3596
Spikelets—seed fert.	0,4663*	0,5562**	0,7593***	—0,1542	0,2257	0,2160	—0,0576	0,3535

that the main part of the depression fall from standard to I_1, observable in the curve, in reality is a fall from standard down to raw polyploid.

The correlation coefficients for various character combinations, calculated on the family means of Table 2, are summarized for the different generations of inbreeding in Table 3. There is an obvious positive correlation between the degrees of vitality in different characters. This correlation is stronger and of a higher statistical significance in the tetraploid material, thereby probably revealing a smaller bulk of genes than that existing in the diploid strain from which it once has taken its origin.

DISCUSSION.

As is evident from the depression curves in this particular case, the depression slope is less steep in the tetraploids. Of more general importance is the fact that evidently the depression slopes are the expressions of different functions. Thereby the confinement to the chromosomes of the factorial background to the inbreeding depression is made probable. This probability is highly increased by the demonstration of the very diverging results from selfing the different components of a diploid-tetraploid sectorial chimaera. Here, both materials have started from the same bulk of genes and degree of heterozygosity. It is difficult to realize how the plasmatic conditions would be able to bring about such divergences (HERIBERT NILSSON, 1937).

From the results it seems justified to judge that at least to the greater part the depression, resulting from inbreeding, is caused by factors confined to the chromosomes. Thereby it is interesting to note that the depression fall of the tetraploids can be quite considerable after one generation of inbreeding, too. RASMUSSON (1933) has sug-

gested that interaction conditions in the addition of related gene effects might influence the depression slope in order to render the effect of inbreeding in the first inbred generation or generations smaller than would be expected on the basis of continued halving of heterozygosity. The slope of the depression curve would therefore become more or less S-shaped. The reduction of the depression fall in the first generations of inbreeding will be more pronounced with increasing number of segregating factors or the stronger the gene interaction tends to be. Results from *Brassica oleracea* (RASMUSSON, 1932) are mentioned as demonstrating such an inbreeding depression.

HALDANE (1930) and FISHER (1949) have given formulas for the rate of approach to homozygosis in selfed autotetraploids. The ultimate rate of decrease of heterozygosis will be 0,83, the corresponding value for the diploids being 0,5. RASMUSSON has given formulas, admittedly not quite accurate, for calculating the average »genotypic value» as contrasted to the »factorial value» of an inbred generation (1933). According to the given formula applying the above-mentioned values to various numbers of polymeric factors and assuming different degrees of interaction, it will be found that the tendency to an S-shaped slope of the depression curve, observable for the diploids already, is much more pronounced for the tetraploids. Assuming 100 segregating factors and an interaction = 0,9 (no interaction, *i. e.* strict additiveness of factors, being = 1), it will be found that the tetraploids have not reached their maximum value of depression fall even after 10 generations of selfing. The inbreeding depression being caused mainly by the less economical distribution of alleles, brought about by the increase of homozygosity following inbreeding, the tetraploids must be expected to be extremely resistant to inbreeding, in every case in the first inbred generations.

There is another possibility of analysing the influence on the progress of inbreeding depression, caused by polymeric factors, where the recessives are indifferent. Assuming a diploid highly inbred population, the homozygous types with different numbers of dominant factors will be distributed according to the Gaussian frequency curve, and one can easily calculate the value for the inbreeding minimum at different numbers of originally segregating factors and at different degrees of factorial interaction. It will be found thereby that the value of the inbreeding minimum is increased with an increasing number of segregating factors or with an increase of the interaction. This increase of the inbreeding minimum will be quite considerable even at a num-

ber of segregating polymeric factors less than five, and at ten segregating factors the inbreeding minimum will differ very little from the value 1. Even if the inbreeding minimum is not calculated on the basis of the number of segregating factors only but on the basis of these + the fundamental genotypic value (RASMUSSON, 1933) it will be found that the considerations hold true to their ultimate effect. Therefore it seems justified to assume that polymeric factors are hardly capable of an essential influence on the progress of the inbreeding depression. An S-shaped depression slope caused by polymeric factors, as a matter of fact, very well might be an ingredient in the big total depression fall, but it is working on a scale too reduced to be perceptible.

SHULL (1948) points out that the cumulative effect of dominant favourable genes cannot be the explanation of hybrid vigour. One might invert this statement. Uneconomical recombination of dominant favourable genes cannot be the cause of inbreeding depression. Regard must be paid to the actively deleterious effect of recessive genes. WRIGHT (1931) calls attention to the fact that the selection conditions for recessive and dominant deleterious mutations should lead to »a marked correlation in nature between recessiveness and deleterious effect».

Now there seems to be a certain tendency to an S-shaped slope of the depression curves. Especially this applies to the curves for the number of spikelets. The material is certainly very meagre, but it should be observed that in this character both the diploid and the tetraploid curves show their greatest deviation from the theoretical curves, based on the assumption of a direct connection between the degree of the depression fall and the continued halving of heterozygosity in each generation of inbreeding.

EAST (1936) has assumed a completing or rather augmenting effect between efficient alleles within a locus. The degree of strict additiveness of allelic effects was assumed to be directly connected with the degree of divergence between the alleles of a locus. On the assumption that the homozygotes are both vital, a model experiment with such alleles will reveal that the value of the inbreeding minimum will be determined only by the degree of superiority of the heterozygote as compared to the homozygotes. On the other hand, different values for factor number or interaction between factors in different loci will influence the slope of the depression curve only within the bounds determined by the superiority of the heterozygotes. A perceptible S-shaped slope of the curve is therefore possible in this case. As in the

case of polymeric factors the tendency to an S-shaped curve is increased in the tetraploids.

The perceptibility of this tendency to an S-shaped slope of the depression curve might be dependent on the part that such factors — may they be really gene alleles or linked gene complexes or chromosomal variants (DOBZHANSKY, 1950) — form in the inbreeding depression as compared to the part caused by the segregation of deleterious recessives, covered by favourable dominant alleles in the heterozygotes. CROW (1948), BRIEGER (1950), and DOBZHANSKY (1950) have presented strong arguments in favour of the assumption that such heterotic gene interaction should be of great importance for the conditions of the vitality. At any rate, a stronger tendency to an S-shaped slope of the depression curve might be expected with an increasing probability that divergent factors have been brought together before the inbreeding. RASMUSSON's results (1932) from inbreeding of F_1 from the cross between common cabbage and Savoy cabbage might be an illustration of this. The presence of such divergent factors is probably less likely when one is dealing with a rigorously selected and highbred population.

The curves make it probable, therefore, that the changed segregation conditions are strongly connected with the diverging effect of inbreeding in the tetraploids. As theoretically could be expected from that fact, the fall of the depression curve of the tetraploid inbred material, when compared to their standard population, is less steep than the corresponding diploid fall. Now it would be of great interest to know, how the inbreeding minimum of diploids and tetraploids will coincide when starting from materials of the same bulk of genes and heterozygosity.

The demands on an exact conformity in these respects can evidently be filled only by a sectorial chimaera. From the formula given by HALDANE (1930) for approach to equilibrium of genes in autopolyploids it is evident that a diploid and a tetraploid population, both at equilibrium, when corresponding to one another in the total proportion of heterozygous allelic combinations, cannot coincide as to the bulk of genes, and conversely. Starting from the same bulk of genes, a tetraploid population having attained its equilibrium will have an essentially higher proportion of heterozygous allelic combinations than will the corresponding diploid population. (This difference, however, will be strongly reduced the more the proportions of the alleles diverge from the ratio 0,5 : 0,5.) These considerations demonstrate the

difficulties in this case of choosing a standard material proper for calculating the depression fall and the inbreeding minimum. Two large I_1-generations from a diploid-tetraploid sectorial chimaera would certainly be quite comparable to one another, but the effect of inbreeding should preferably be calculated with the material of origin, *i. e.* the different parts of the chimaeric plant, taken as the standard. This not conveniently done, in this particular case among other things caused by the material being annual, there remains only to choose common élite population as a standard. In the present material, however, as has become evident from the foregoing considerations concerning the low number of diploid kernels forming the basis of the tetraploid population, one has lost the control of conformity to a very high degree. It seems impossible, then, to judge from the present materials which material will be the most sensitive to inbreeding at comparable stages of reduced heterozygosity. It should not be unexpected, however, if, in the long run, the tetraploids would be more sensitive, this being caused by the high frequency of inferior aneuploids in population material of Steel-rye. MÜNTZING (1951) has found 20—25 % aneuploids in population material of Steel-rye, and my own (unpublished) results agree, at the same time perhaps indicating an increase of the frequency of aneuploids with increasing degree of inbreeding. This demonstrates the desirability of a control of the chromosome numbers, more intense than has so far been possible in the present tetraploid material.

SUMMARY.

Results from the four first generations of inbreeding (selfing) from diploid and corresponding tetraploid populations of rye are presented. These are completed with results from I_1 and I_2 from diploid-tetraploid sectorial chimaeras of the same variety. A change of the function for the depression slope after attainment of polyploidy is evidently obtained. This indicates that the factors, determining the inbreeding depression, are at least predominantly connected with the chromosomes. A pronounced depression fall also in the tetraploids might indicate the comparative insignificance of polymeric cumulative factors for the inbreeding depression. From the present materials it has not been possible to determine which material will in the long run be the most sensitive to inbreeding.

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ON POSITION CORRELATIONS OF UNIVALENTS AND QUASI-BIVALENTS FORMED BY STICKY UNIVALENTS

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AS is well-known, the unpaired chromosomes or univalents of the first division of meiosis behave in a characteristic manner which is quite different both from the behaviour of paired chromosomes and from that of ordinary mitotic chromosomes. While the bivalents arrange themselves regularly on an equatorial plate during metaphase the univalents usually remain distributed at random over the spindle. This expression »at random», which often has been used in this connection, is obviously only an approximation. It is clear that certain positions of the univalents are more common than others and that there are differences between different materials in this respect. As a rule, the univalents are not lying in the central parts of the spindle body but they are distributed over the surface of the spindle, in some materials more close to the poles and in others more close to the equator.

The position of the univalents at metaphase is influenced by various factors some of which are known. It is possible to speak of position correlations of the univalents. Thus it appears that when there are *no bivalents or only few* of them, then the univalents show a more polar distribution at metaphase, while they show a more equatorial distribution in cells having many bivalents (ÖSTERGREN, 1949). The position of the univalents may also be correlated to the *structure of the meiotic chromosomes*; in *Tulipa*, where the kinetic constriction is unusually pronounced (resemblance to ordinary mitosis) the univalents show a stronger tendency towards equatorial arrangement than is usually the case in other materials (ÖSTERGREN, 1951). It is a well-known fact that the position of the univalents is also correlated to the *stage of development* of the cells; at late metaphase they often accumulate in the equatorial spindle region. Furthermore, the univalents are in some cases not arranged independently of one another at metaphase, but they show

a *mutual dependence* of their positions in a special manner discovered by RIBBANDS (1937). The present paper is devoted to a study of this last kind of position correlation and some related effects. During the course of our study we found a peculiar kind of »quasi-bivalents» formed by sticky univalents, and as these configurations have an obvious effect on the distribution of the univalents on the spindle they will also be treated here.

RIBBANDS (1937), who studied meiosis in the anthers of the hybrid *Lilium candidum* × *chalcedonicum* (*L.* »*testaceum*»), states that a couple of univalents belonging to the same chromosome pair usually lie with their kinetochores on the same spindle arc. This holds either the univalents are lying on opposite sides of the equator or on the same side, *i. e.* they may lie closely approached to one another or separated by a great distance. We have made a re-investigation of this effect and we are able to confirm RIBBANDS's conclusion in principle. But we would not state that the univalents use to have their kinetochores on *the same* spindle arc. As a rule, they are lying on different spindle arcs, but there is a statistically significant tendency of these spindle arcs to be more close to one another than expected from random distribution. The univalents thus show an obvious tendency to lie on spindle arcs closely approached to one another.

Three different materials were used in the present study. The first one is a garden hybrid of *Lilium* called *L.* »*testaceum*» and generally considered to be *L. candidum* × *chalcedonicum*. The anthers containing p. m. c.'s at meiosis were cut in small pieces and fixed in chrome-acetic-formalin (NAVASHIN's fixative as modified by MÜNTZING, 1933). The other materials were two plants of *Secale cereale* (the variety »Östgöta Gråråg»). The first plant was a *partially asynaptic* one and contained two B chromosomes of the »large iso-fragment» type (MÜNTZING, 1945; MÜNTZING and LIMA-DE-FARIA, 1949). The second rye plant also contained two B chromosomes of the same type, but it showed a normal chromosome pairing, only the B chromosomes being often unpaired. These two plants will be denoted by *Secale cereale* plant nr. 1 and plant nr. 2 in the present paper. The anthers of the rye plants were fixed in chrome-acetic-formalin after prefixation in acetic alcohol. All the material was embedded in paraffin and cut into microtome sections. The slides were stained with crystal violet and mounted with Canada balsam. The slides of *Lilium* and the first rye plant were earlier used in the study of ÖSTERGREN (1951). The slides of the second rye material were borrowed from Professor MÜNTZING, whose kind

help in this respect is gratefully acknowledged. At the study of the slides we used both ordinary microscope illumination and phase contrast. When phase contrast illumination is used the details of the unstained cytoplasm and spindle stand out very clearly and an observation of the position of the chromosomes on the spindle is much facilitated (Fig. 1).

According to RIBBANDS (1937), the number of pairs of univalents per cell in *L.* »*testaceum*» varies from 0 to 3 with an average of 0,83 pairs per cell. In the cells with complete pairing there are 12 bivalents. We have not counted the frequency of univalents in our slides, but in any case the chromosome behaviour does not seem to be very different from that observed by RIBBANDS. The rye material used by us contained 7 chromosome pairs belonging to the normal complement and one pair of B chromosomes of the »large iso-fragment» type. This chromosome type is an iso-chromosome derived by duplication of the long arm of the standard type of B chromosome [1] found in rye (MÜNTZING and LIMA-DE-FARIA, 1949; MÜNTZING, 1951). These iso-chromosomes may pair with one another, or they may pair within themselves to form ring-shaped univalents, or they may finally be completely unpaired having the shape of ordinary univalents (MÜNTZING, 1951). The chromosome pairing in our material of plant nr. 1 varies from $1_{II} + 14_{I}$ to 8_{II} with an average of $5{,}9_{II} + 4{,}2_{I}$. The iso-chromosomes have a stronger tendency to form univalents than the normal chromosomes, but it is difficult to get quantitative data in this respect, since they are often so similar to the normal chromosomes that they cannot be recognized. It is a characteristic feature of these iso-chromosomes that they often form univalents even in otherwise normal plants (MÜNTZING, *l. c.*). Rye plant nr. 2 of our material shows normal pairing of the chromosomes of the normal complement but the two B chromosomes usually form univalents.

[1] The B chromosomes of rye are a special chromosome type which is not homologous with any chromosome region of the normal complement. They pair only with one another but not with the normal complement. They occur as supernumeraries in a low frequency of plants in many rye populations. Their origin is unknown but they seem to be a very old type since the same or closely related types can be found in rye varieties from widely separated geographical regions (MÜNTZING, 1945, 1949, 1950). (The idea earlier expressed by MÜNTZING, 1943, that they were frequently produced anew from the normal chromosomes was abandoned in later publications.) These B chromosomes are also called the »standard fragment» of rye, an unfortunate term, since it easily leads to the misunderstanding that they originate by simple fragmentation from the normal complement.

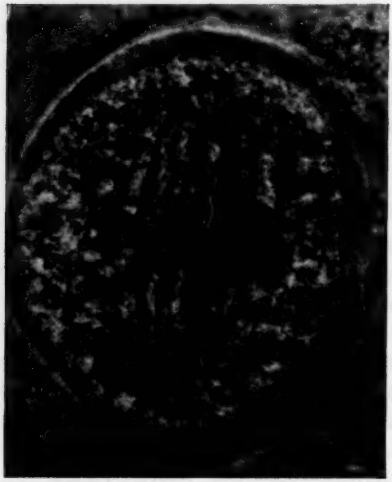


Fig. 1. Photograph of a first metaphase cell of *Lilium candidum* × *chalcedonicum*. By means of phase contrast illumination the structural details of the unstained spindle and cytoplasm are made visible. It can be seen that the univalent on the right side just above the metaphase plate is attached to chromosomal spindle fibres. These are also very distinct at the bivalent on the left side of the plate. The other univalent is slightly out of focus in the lower left region. (The chromosomes are stained with crystal violet and the preparation is mounted in Canada balsam.) — × 1200.

One purpose of the present study was to investigate the position correlations which univalents belonging to the same chromosome pair show relative to one another. When there are more than two univalents in a cell, however, it is often difficult to find out how they belong together in pairs. To eliminate this difficulty we selected cells which showed a single pair of univalents only, when we collected the data of Table 1 presented further below. (An exception to this is represented by a cell of *Lilium* with four univalents, where the two pairs were distinctly different from one another.)

The investigation is much facilitated by the fact that the univalents studied always were lying on the *surface* of the spindle. This holds either they were lying in the equator or in various other positions on the spindle. The univalents also always have their kinetochores directed inwards towards the spindle axis and their chromosome arms outwards. This demonstrates that the univalents are subjected to the mitotic forces in the same way as other chromosomes, their kinetochores are attracted to the spindle poles and the spindle strives to extrude the chromosome body out of it (ÖSTERGREN, 1951).

The univalent pairs were classified into two main groups with respect to their position on the spindle, *viz.* such where the kinetochores of the two univalents were lying on spindle arcs closely approached to one another and such where the spindle arcs were more

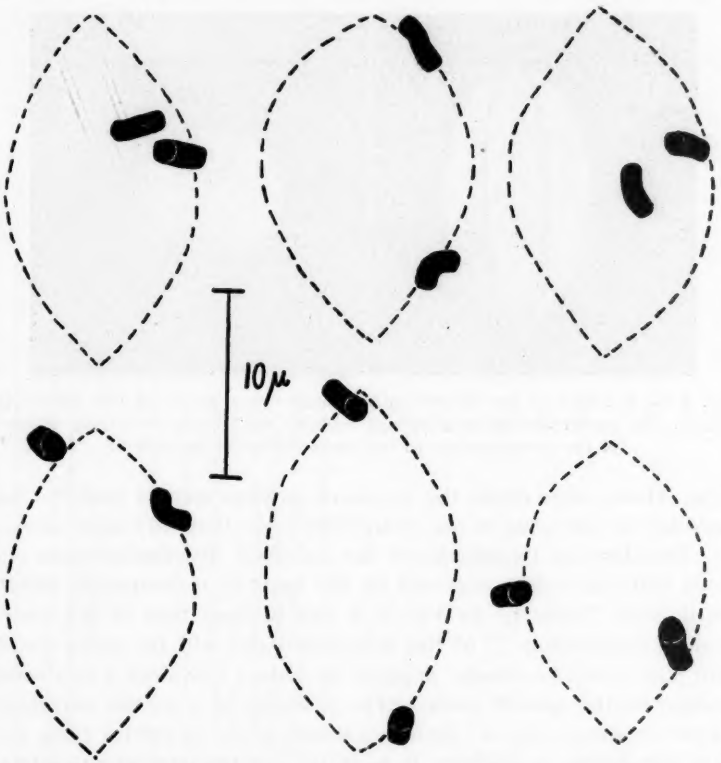
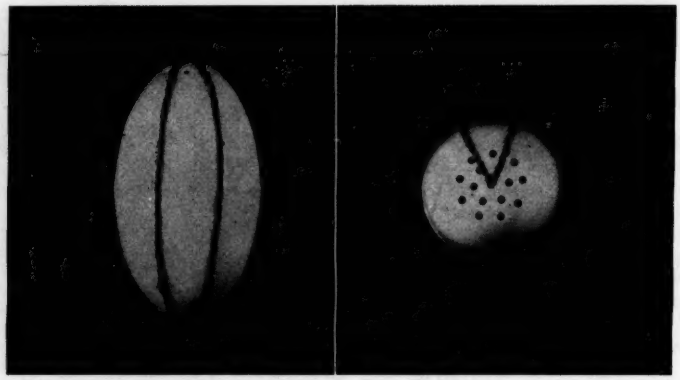


Fig. 2. *Secale cereale*, $2n = 14 + 2$ »large iso-fragments», a partially asynaptic plant. Some examples showing the position on the spindle of pairs of univalents at first metaphase. — × 2800.

separated (Table 1). The limit between the two groups was put at an angular separation of the spindle arcs of 45° ($^1/_8$ of the circumference of the cross section of the spindle). Within these two main groups, the univalent pairs were further classified in such cases where both members were lying on the same side of the equator, such where they were on different sides, and such where one or both of them were located in the equator (Table 1).

In order to make the classification more easy the cells were compared with a model of a spindle on which the critical 45° region had been marked (Figs. 3—4). When necessary the height distances in the cell were estimated by means of the micrometer screw of the micro-



Figs. 3—4. A model of the mitotic spindle made from pieces of two plastic salt-shakers. The region marked with strings contains the spindle arcs lying within $^1/_8$ of the circumference of the cross section of the spindle.

scope. Those cases where the univalent position seemed doubtful were classified as belonging to the group with more than 45° separation.

The observed frequencies of the univalent distribution were compared with the values expected on the basis of a completely random distribution (Table 1). In Fig. 5 it can be seen that in the case of random distribution $^1/_4$ of the univalent pairs will be inside the 45° limit and $^3/_4$ will be outside. Suppose we have a univalent *A* in a certain position on the spindle surface. The positions of a second univalent *B* which are inside the 45° limit (measured from *A*) extend from B_1 to B_2 in Fig. 5. The range from B_1 to B_2 is $^1/_4$ of the total spindle surface.

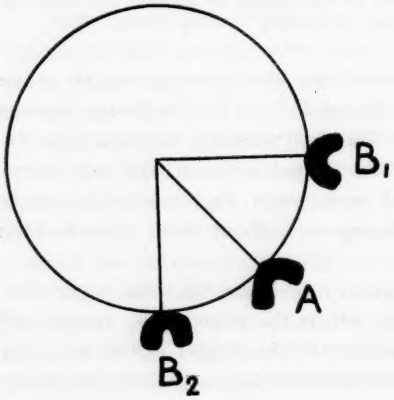


Fig. 5. A figure explaining the probability on the basis of mere randomness for a pair of univalents to fall on spindle arcs lying within the same $^1/_8$ of the cross section of the spindle. See text.

TABLE 1. ***Position of the kinetochores of univalent pairs at first metaphase.***

Material		Number of pairs of univalents showing an angular separation of their kinetochores when projected on a cross section of the spindle						Total number of pairs	χ^2 and significance
		less than 45°			more than 45°				
		Kinetochores on the same side of the equator	Kinetochores on opposite sides of the equator	One or both kinetochores in the equator	Kinetochores on the same side of the equator	Kinetochores on opposite sides of the equator	One or both kinetochores in the equator		
Lilium candidum × *chalcedonicum*	Observed	7	7	4	14	8	8	48	$\chi^2 = 4{,}00$ $0{,}05 > p > 0{,}02$
	Sum	18			30				
	Expected 1 : 3	12			36				
Secale cereale (Plant nr. 1)	Observed	9	7	6	10	12	10	54	$\chi^2 = 7{,}13$ $0{,}01 > p > 0{,}001$
	Sum	22			32				
	Expected 1 : 3	13,5			40,5				
Sum for both materials	Observed	40			62			102	$\chi^2 = 10{,}99$ $p < 0{,}001$
	Expected 1 : 3	25,5			76,5				

χ^2 for heterogeneity = 0,14 (quite insignificant).

It can be seen in Table 1 that there is a satisfactory statistical significance for the existence of a non-random distribution of the univalents. Univalents belonging to the same pair are preferentially located on spindle arcs close to one another.

In Table 1 the chromosome pairs were also classified in such where both members are on the same side of the equator, such where they are on opposite sides, and such cases where one or both of them lie in the equator. If the distribution had been at random in this respect, one should expect that the two univalents would just as often be lying on the same side of the equator as on opposite sides of it. A test with the χ^2 method demonstrates that the observed values agree well with what is expected on the basis of complete randomness (χ^2 for the whole material $= 0{,}486$, p *ca.* $0{,}5$). There is no difference in this respect between the cases where the univalents lie on neighbouring spindle arcs and those where they lie on remote ones.

It was, however, considered that this distribution of the univalents on the two spindle halves should be subjected to a critical test by a study of a larger material. At this study the rather troublesome classification of the cells with regard to the 45° limit was omitted and they were simply classified with regard to their position on the two spindle halves, the univalents lying either on the same side or on opposite sides of the equator (Table 2). This study which demonstrated the existence of significant deviations from random distribution, will be discussed in more detail further below.

What is the cause of the preferential distribution of the univalents on spindle arcs closely approached to one another (the effect demonstrated by Table 1)? This arrangement is easily understood as a relic from a side-by-side arrangement of the homologous chromosomes during the preceding prophase. It was observed by RIBBANDS (1937) in *Lilium* »*testaceum*» that the univalents belonging to a chromosome pair used to lie arranged in pairs during diplotene, diakinesis and even prometaphase (*l. c.*, p. 5). Such an arrangement seems to be of a quite common occurrence in the case of chromosomes which have been wholly or partially associated during pachytene but failed to form chiasmata. It was also observed by PRAKKEN (1943) in asynaptic *Secale cereale* and he makes a review of other similar cases (*l. c.*, p. 489).

According to the theory of co-orientation of ÖSTERGREN (1951), the univalents show active movements on the spindle during the prometaphase and metaphase. These movements are produced by attractions of their kinetochores to the spindle poles. The direction of

the force may change from one pole to the other depending on changes in the kinetochore orientation. Thus, there should result irregular movements to and fro of the univalents in the two opposite poleward directions. As the univalents originally are arranged side by side in the prophase these movements to and fro will take place along spindle arcs close to one another, and a metaphase arrangement of the kind observed here will result.

Why is it then found that the univalents sometimes lie on spindle arcs more widely separated? There can be given some reasons for this fact. Firstly, there are, of course, cases when they have failed to be arranged in pairs at the prophase. Secondly, there is also another factor of importance. The univalents use to lie on the surface of the spindle during metaphase. This means that they often have to move out of the central parts of the spindle in the transverse direction during prometaphase. When they lie close to one another it is, of course, probable that these transverse movements usually will be directed to the same region of the spindle surface. If their original position, however, was in the very centre of the spindle, as it sometimes should be, then it might happen that the univalents pass as easily to opposite regions of the surface as to neighbouring ones. Furthermore, the other chromosomes present in the same prometaphase may obstruct the movements of the univalents and force them to pass to regions of the spindle surface more remote from one another.

In this connection it should be pointed out that the explanation of this position correlation effect, which was originally offered by RIBBANDS (1937), is quite closely related to the one given above. He also suggested that the arrangement at metaphase was due to a movement apart in the longitudinal direction of the spindle performed by univalents arranged in pairs at the preceding prophase. Strangely enough, RIBBANDS reports that of the univalents on opposite sides of the equator as many as 83 % are arranged on the »same arc of the spindle», an expression which seems to imply a very liberal interpretation of what can be considered as *the same* arc. His methods, however, did not permit a good observation of the spindle and he has not drawn the spindle in his figures. Also it would seem that he has overlooked the fact that the univalents lie on the spindle *surface* even when they are not in the equator.

As already mentioned above, the distribution of the univalents on the two halves of the spindle was investigated on a bigger material. In this case all cells were discarded which had univalents in the equator.

TABLE 2. *Distribution of the members of univalent pairs on the two halves of the spindle.*

Material	Sample Nr.	Univalent distribution 1:1	Univalent distribution 2:0	χ^2 for deviation from equal frequency	Probability
Secale cereale Nr. 1	1	57	36	4,74	$0{,}05 > p > 0{,}02$
	2	36	28	1,00	$0{,}5 > p > 0{,}3$
	3	32	21	2,28	$0{,}2 > p > 0{,}1$
	Sum	125	85	7,62	$0{,}01 > p > 0{,}001$
Secale cereale Nr. 2	4	90	116	3,28	$0{,}1 > p > 0{,}05$
	5	62	41	4,28	$0{,}05 > p > 0{,}02$
	6	136	106	3,72	$0{,}1 > p > 0{,}05$
	Sum	288	263	1,13	$0{,}3 > p > 0{,}2$
	Total	413	348	5,55	$0{,}02 > p > 0{,}01$

	Degrees of freedom	χ^2	Probability
A. Deviation from equal frequency of all the individual samples	6	19,31	$0{,}01 > p > 0{,}001$
B. Heterogeneity between all the samples of the material	5	13,76	$0{,}02 > p > 0{,}01$
C. Heterogeneity between plants nr. 1 and 2	1	3,20	$0{,}1 > p > 0{,}05$
D. » » samples within plants	4	10,55	$0{,}05 > p > 0{,}02$
E. » » » » plant nr. 1	2	0,41	$0{,}9 > p > 0{,}8$
F. » » » » » » 2	2	10,15	$0{,}01 > p > 0{,}001$

The study was first made on the rye plant nr. 1, but as there was obtained a significant deviation from random distribution it was considered worth while to continue the study, and plant nr. 2 was also taken up for investigation.

The main results of this study can be seen in Table 2 which records the observations made on cells containing a single pair of univalents. The distribution called 1 : 1 in the table represents the cases having one univalent on each side of the equator, and 2 : 0 means the cases where both univalents lie on the same side. On the basis of random occurrence both these distributions would appear with equal frequency. In the material from plant nr. 1 the three samples (1—3 in Table 2) represent material from three different slides. The material from the first two slides corresponds to one anther each and the third slide contains material from three anthers. The three samples from plant nr. 2 (4—6 in the table) are all from one single anther. The

division in three samples in this case originated in the following way: First the cells were classified until it was considered that a sufficient number of observations had been collected. The result (sample nr. 4) indicated, however, that there might actually be a deviation from randomness in the somewhat unexpected way of an excess of 2 : 0 cases. To get clear information on this question some more cells in the same slide were then studied (sample nr. 5). This sample indicated fairly strongly a real deviation in the quite opposite direction, for which reason the material was extended by a study of a second slide from the same anther (sample nr. 6).

The statistical treatment (summarized under Table 2) gives the following result:

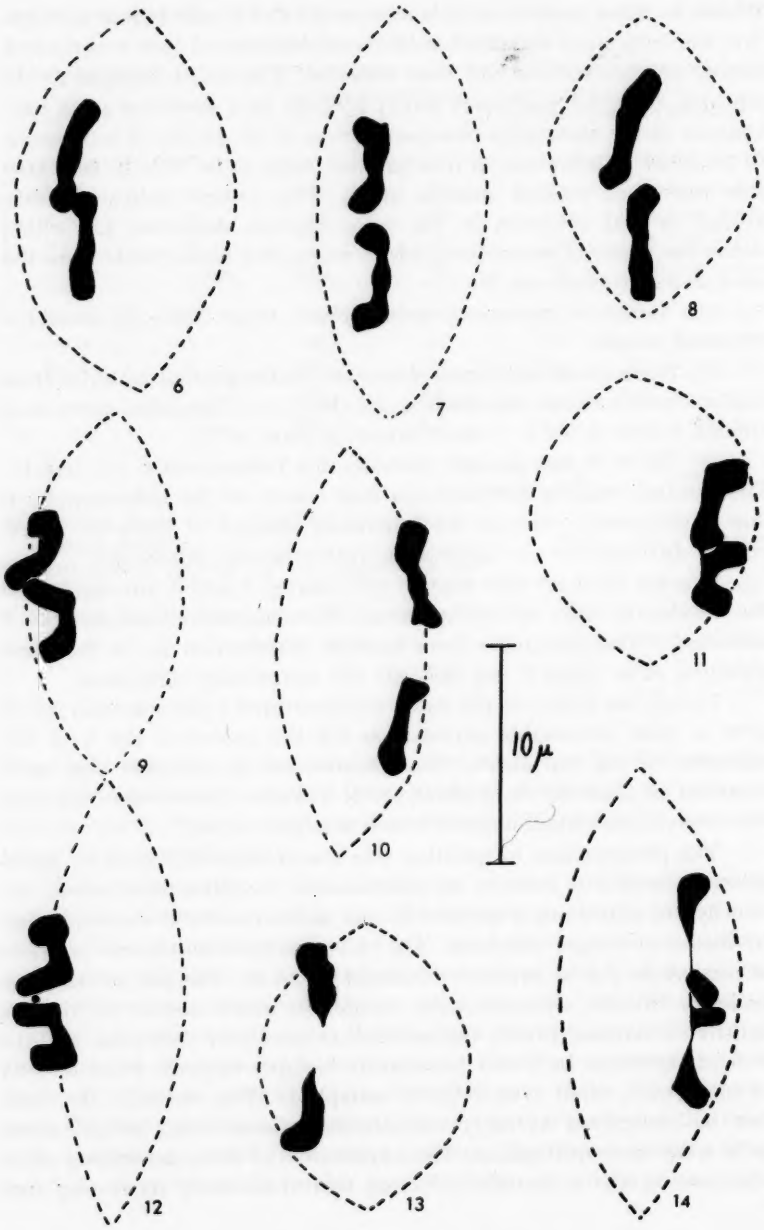
(1) There occur significant deviations in the present material from random chromosome distribution (*cf.* line A). Especially, there is a notable excess of the 1 : 1 distribution in plant nr. 1.

(2) There is also distinct evidence for heterogeneity (*cf.* line B). The detailed analysis demonstrates that nearly all the heterogeneity is due to differences between the individual samples of plant nr. 2, *i. e.* cells in different *regions* of the same anther behave differently.

In plant nr. 1 we also studied cells having 4 and 6 univalents, but the number of cells was rather small, 92 cases with 4 and 30 with 6 univalents. The deviations from random distribution go in the same direction as in Table 2, but they are not statistically significant.

During the course of the study we discovered a phenomenon which gives a quite reasonable explanation for the excess of the 1 : 1 distribution of the univalents. The phenomenon in question was quite common in plant nr. 1. In plant nr. 2, however, there were only very few cases of this kind, none of which was quite clear.

The phenomenon in question was found especially in early metaphase cells and it consists of chromosome configurations which obviously are univalents associated to one another without chiasmata but by means of simple stickiness. The two associated univalents co-orient at metaphase just as ordinary bivalents use to do. The pull of the kinetochores towards opposite poles (which is characteristic of the co-oriented condition) breaks the association caused by stickiness, and the two partners can be found to separate towards opposite poles already at metaphase, often even at early metaphase (Figs. 6—12). The stage can be recognized as early metaphase, because many neighbouring cells were in prometaphase. The appearance of these separating chromosomes is also quite different from that of ordinary separating ana-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phase chromosomes. In ordinary first anaphase chromosomes there can be seen a clear doubleness. The two chromatids separate more or less from one another when the anaphase starts. It is probably this change, »the lapse of chromatid association», which initiates the anaphase separation. The change takes place at the same time in both paired and unpaired chromosomes (*cf.* ÖSTERGREN, 1951, p. 99). This doubleness is especially clear (even in early anaphase) in such chromosome regions which have been lying distally of a chiasma at metaphase, a natural consequence of the partner exchange at the chiasma. The chromosomes considered as sticky univalents separating from one another do not show traces of such a chromatid separation, however. The only reasonable interpretation of these cases is that the chromosomes have been associated simply by stickiness, and that they have been separated by the poleward pull working on their kinetochores.

The shape of these chromosomes often demonstrates that they have been subjected to a mechanical extension between their kinetochores and the point where they have stuck together. They are often bent both at the kinetochores and at the association regions, a consequence of the unequal extension of the different sides of the chromosome body due to the unilateral attachment of the pulling force on the chromosome.

Some stickiness is probably an often occurring property of chromosomes and it is quite natural that the partner chromosomes of asynaptic bivalents may happen to stick together, intimately associated by mutual coiling (torsion pairing) as they often are during the prophase. According to the theory of co-orientation (ÖSTERGREN, 1951), chromosomes which are associated with one another will co-orient, irrespective of whether the association is due to chiasmata or some other mechanism. The observed chromosome behaviour is quite in accordance with the theoretical expectations.

Figs. 6—14. *Secale cereale*, $2n = 14 + 2$ »large iso-fragments», a partially asynaptic plant. Quasi-bivalents (Figs. 6—12) and ordinary bivalents (Figs. 13—14) at first metaphase. The quasi-bivalents of Figs. 6—10 are from early metaphase, those of Figs. 11—12 from late. They show various stages of separation of the univalents which have been associated by stickiness. In Fig. 11 the chromosomes may stick together at non-corresponding regions. In Fig. 12 there is a small droplet, presumably of fluid matrix, between the two separating chromosomes. Fig. 13 shows an ordinary bivalent where the terminal connection between the partners is attenuated to a thin thread. Fig. 14 may be a quasi-bivalent, or it may be an ordinary bivalent subjected to a strong mechanical deformation by the kinetochore forces. — × 3300.

Observations on first anaphase in the rye plant nr. 1 also give evidence of a certain degree of stickiness of the chromosomes. Bridge formations of the sticky type are not rare at early anaphase. They are no severe obstacle to the chromosome separation and disappear at late anaphase.

It would be interesting to know if these chromosomes that separate from one another already at early metaphase remain lying at opposite poles during the rest of the division or if they later may start to behave as ordinary univalents. Unfortunately, our material cannot answer this question. The best way to investigate it would probably be a study of the chromosome behaviour in a living cell. In any case, the chromosomes which have been paired by means of stickiness and separated already at metaphase will cause an excess of 1 : 1 cases in the distribution of the univalents. It is also clear that they will contribute to the cases when two univalents are found to lie on spindle arcs very close to one another but on opposite sides of the equator.

If a term is desired for these chromosome pairs associated by means of stickiness, it may be suitable to call them »quasi-bivalents». The term »pseudo-bivalent» has earlier been used by LEVAN (1937) for quite another kind of chromosome configuration, *viz.* for chromosomes in an ordinary mitosis which are associated by means of a chromatid interchange (called a »pseudo-chiasma» by LEVAN, a term which he a few years later, however, is using for quite a different effect; LEVAN and TJIO, 1948).

It is of common occurrence (in the most various materials) that chromosome connections at terminal chiasmata may be drawn out to thin threads which sometimes even may be invisible (*e. g.*, Fig. 13). Such bivalents should scarcely be considered as cases of precocious chromosome separation like the quasi-bivalents. Most probably the connection, although thin, will remain unbroken until the start of anaphase. During a study of univalents one must be cautious not to confuse bivalents of this kind with a pair of univalents.

One may occasionally meet the interpretation in the literature that univalents lying close to one another at metaphase do so because of stickiness. This does not appear probable. From the theory of co-orientation (ÖSTERGREN, 1951) one should expect that any factor (like stickiness or chiasmata) tying two chromosomes to one another will cause them to co-orient and become pulled towards opposite poles.

As already mentioned above, there was a great difference in the frequency of quasi-bivalents between the rye plants nr. 1 and nr. 2.

These configurations were common in plant nr. 1 but in plant nr. 2 there were scarcely any certain cases. The following comments may be made to this difference. (1) The excess of the 1 : 1 distribution of the univalents was also stronger in plant nr. 1 than in nr. 2, just as expected if the quasi-bivalents were the cause of this excess. (2) There appeared also to be less stickiness in the anaphases of plant nr. 2. (3) The frequency of univalents was smaller in plant nr. 2, and as quasi-bivalents are formed by univalents, their frequency should also for this reason be smaller. (4) In plant nr. 2 all the univalents were formed by B chromosomes (of iso-chromosome type) and these chromosomes should be less intimately associated with one another by torsion pairing at the prophase than ordinary A chromosome univalents use to be. In the iso-chromosomes much of the pairing affinity is satisfied by inter-arm pairing so there will be less inter-chromosome pairing. This should also reduce the frequency of quasi-bivalents. (5) Only late metaphase was studied in this plant and many quasi-bivalents may already have separated, especially as one should expect them to separate earlier and more easily when the stickiness is weak. — The tendencies to an excessive 1 : 1 distribution in plant nr. 2 are not strong and it is not unreasonable to suppose that they are produced by the same stickiness — quasi-bivalent mechanism as found in plant nr. 1. It is clear that such properties as stickiness, quasi-bivalent formation and non-random univalent distribution are properties that will vary from one material to another and naturally comparisons of more materials in these respects should be valuable.

The heterogeneity found within a single anther of the rye plant nr. 2 presents an intricate problem. The effect would seem to imply that the univalents behave in a different way in different regions of an anther, presumably because of local physiological differences. It is naturally well conceivable that local physiological conditions may influence the degree of stickiness, leading to a stronger excess of the 1 : 1 distribution in one part of the anther than in another. The whole material of this anther, however, deviates but little from the expectation on the basis of randomness. This makes us suspect that there may also be another effect operating in this material, an effect favouring a 2 : 0 distribution. In some regions the one effect may prevail, in others the other one, and in the total material the two effects cancel one another.

Thus, the question is raised concerning the nature of an effect leading to a preference of a 2 : 0 distribution of a pair of univalents. The following suggestions may be made:

(1) So-called »secondary pairing» of univalents. Various authors have suggested that in their materials an association of univalents at metaphase may be due to secondary pairing, *i. e.* to long distance attraction forces between homologous chromosome regions, a kind of attraction related to the one producing the prophase pairing of homologous chromomeres in the early meiotic stages. This interpretation seems rather dubious for various reasons. *Firstly*, the very existence of long range attraction forces capable of producing secondary and somatic pairing seems rather doubtful. When somatic pairing is found at metaphase it is probably a relic from a more close pairing or association in earlier stages such as resting stage and prophase (*e. g.*, COOPER, 1948). *Secondly*, if there had been such attraction forces between the univalents, the pull by which they would influence one another should have produced the same effect as the mutual pull working between paired chromosomes during the co-orientation (ÖSTERGREN, 1951). The univalents would then turn their kinetochores towards opposite poles and they would separate in the two opposite poleward directions, moved by the kinetochore forces. Thus, the forces of secondary pairing would not cause an association of the univalents but instead the opposite effect, *viz.* a separation of them towards opposite spindle poles.

(2) An asymmetry of the spindle. It is quite possible that the first metaphase spindles may be somewhat asymmetrical; their two halves will then not interact in an identical way with the kinetochores of the univalents, the one half will have a greater chance of carrying a univalent than the other one. It is obvious that this half will then also have a greater chance of carrying the second univalent, and there will result an excess of the 2 : 0 distribution.

(3) It is possible that the presence of a univalent in one spindle half induces an asymmetry of the spindle which causes an influence on the second univalent of such a kind that it will be preferentially located in the same spindle half. There is some evidence that the spindle mechanism working on one chromosome is influenced by the spindle action on other chromosomes, *e. g.* the correlation of the displacements of bivalents and trivalents observed in *Anthoxanthum aristatum* (ÖSTERGREN, 1945).

Finally, it should be mentioned that RIBBANDS (1937) made some observations concerning the distribution of the univalents on the spindle halves in *Lilium* »*testaceum*». His data are in good agreement with the expectations from complete randomness, and we have not made any effort to test them again on our *Lilium* material.

Even though the present material gives some evidence for deviations from random distribution of the univalents it is still possible to state that it supports the opinion of ÖSTERGREN (1951) that there is no such interaction between the univalents as is found between the partners of bivalents. The interaction in the bivalents leads to strong forces striving to separate the partners towards opposite poles. Even univalents lying on neighbouring spindle arcs can, however, often be found to lie on the same side of the equator (Table 1). There seem also to be materials where the univalents show a completely random distribution in this respect (*Lilium* »*testaceum*»; *cf.* RIBBANDS, 1937).

There have been presented ideas by various authors that univalents may sometimes associate at metaphase because of stickiness or secondary pairing or that they may also separate owing to mutual repulsion. These opinions should usually be considered with a great deal of caution and scepticism. It is obvious that even on the basis of complete randomness the univalents should sometimes lie close to one another and sometimes more widely separated. In the present paper we have put forward good reasons for the opinion that neither stickiness nor secondary attraction can produce an association of univalents of such a kind that they continue to behave like univalents.

It is clear that there remains much work to be done, and many puzzling problems to be solved, before a satisfactory knowledge has been gained of many aspects of univalent behaviour. The best attitude of approaching the work will be a cheerful optimism that diligent work combined with critical judgement and constructive imagination will gradually unveil more and more of the secrets of nature.

SUMMARY.

A study of the arrangement of univalents belonging to the same chromosome pair demonstrates that these are preferentially distributed on spindle arcs close to one another. This is explicable as a relic from an arrangement in pairs of the univalents in the preceding prophase. The material studied was *Secale cereale* with B chromosomes and *Lilium candidum* × *chalcedonicum*. In rye there is evidence for a non-random distribution of the univalents on the two spindle halves; they tend to move to opposite halves. This is produced by a co-orientation of »quasi-bivalents» formed by sticky univalents. The partners of these »quasi-bivalents» separate already at metaphase. In one plant there may also besides this mechanism exist another one tending

to produce the opposite effect. It is supposed that this may be due to an asymmetry of the spindle of some kind. There are good reasons to believe that the forces of »secondary pairing» will not be able to cause an association of univalents at metaphase.

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HOW TO BREED KENTUCKY BLUEGRASS, POA PRATENSIS L.

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THE genus *Poa* belongs to the grass family and holds about 250 species distributed to all parts of the world. Only one of these species, *i. e. Poa pratensis*, is important as a cultivated plant. In northern Europe we have, however, a limited cultivation of *Poa palustris* and *trivialis* just as in North America of several species, out of which *Poa ampla* from the prairies in the northwestern U. S. A. and *Poa arachnifera* from the Middle West ought to be mentioned. A cosmopolitic species is *Poa compressa*, which is cultivated in some countries.

Sexual as well as apomictic *Poa* species occur. The apomictic *Poa pratensis* develops its E. S's in an aposporous way just as most of the cultivated bluegrass species indigenous to the U. S. A. *Poa palustris*, on the other hand, is diplosporous in conformity to the well investigated *Poa alpina* (*cf.* GUSTAFSSON, 1946—47, pp. 313—314). In the aposporous types the E. S. can be formed either by an archespore cell or by some other cell located close to the archespore in the nucellus as well. This means that usually more than one E. S. is developed in the nucellus, a condition which may cause the origin of twins, triplets, and so on. An important fact is that the chromosome number in the aposporous sacs is unreduced, while it is reduced in the sexual sac, which originates from the archespore cell. The egg cell in the aposporous E. S's forms an embryo without fertilization as a contrast to the egg cell in a sexual sac, which needs fertilization. Most Kentucky bluegrass strains develop one sexual as well as one or more aposporous sacs in the same nucellus. As a rule, the nutrition is not sufficient for several sacs to be completed in the same nucellus, however. Therefore, there will be a competition between different sacs, and usually one of the aposporous sacs is the most successful one in this competition.

It was mentioned that the egg cell in an aposporous E. S. develops autonomously into an embryo. This being usually the case, there is nothing that prevents the egg cell sometimes from being fertilized. The point when the division starts in the egg cell is very important for the

chance of a fertilization, however. If this division starts simultaneously with the E. S. being completed, the chance of fertilization is very small, while on the other hand a late first egg cell division gives good chances for a fertilization.

Even if *Poa pratensis* is aposporous and is able to form embryos without fertilization, a sexual process is needed for the formation of seeds. By investigations during the last decades it has been proved that *Poa pratensis* does not develop any endosperm without a fertilization of the central nucleus in the E. S. (*cf.* NYGREN, 1950, for literature). This is important to know, because a Kentucky bluegrass strain with poor pollen will never give a good yield of seeds. It is a matter of fact that pollen even from other *Poa* species than *pratensis* are useful in this respect (*cf.* ÅKERBERG, 1936 a, p. 246), but this lacks practical importance.

Attention is called to the fact that the breeding of Kentucky bluegrass earlier usually was managed in that way that new strains were built up from single, selected plants or from groups of such plants, which usually had been collected in pastures. In this way many good strains have been raised, some of which have been cultivated in extensive areas.

The only attempt in Europe to cross *Poa pratensis* with another species in the genus is reported by ÅKERBERG (1936 b, 1942), who made the hybrid with *Poa alpina*, while in the U. S. A. the cross between *Poa arachnifera* and *pratensis* was produced already in 1908 by OLIVER (Yearbook of Agr., 1937, p. 1060). During the last decade an extensive breeding program for *Poa* has been taken up by the Carnegie Institution of Washington at Stanford, California (*cf.* CLAUSEN *et alii*, 1949, 1951). In the Stanford experiments up to now crosses have mainly been made between four West American species of *Poa* on one hand and *pratensis* on the other hand. Material for the crosses has been obtained from the Soil Conservation Service as well as from selected plants picked in nature. As a main result of the crossing experiments it is clear that the hybrids, which deviate morphologically from the parents in the cross, in the second generation in most cases give segregating offspring. In the third, fourth, or sometimes in a still later generation the apomictic way of reproduction is rebuilt. In other words, the apomictic, non-segregating way of reproduction in *Poa pratensis* has been broken in the first hybrid generation, after which the genes from the parents have been recombined during one or several sexual generations, whereupon the apomictic way of reproduction is restored in a later generation. These

results fit very well in the scheme found in nature as far as arctic *Poae* concerns (*cf.* NYGREN, 1950). Other experimental data, which point in the same direction, have been obtained in crosses between *Poa pratensis* and *alpina* (ÅKERBERG and BINGEFORS, 1953) as well as in crosses between the diplosporous *Calamagrostis purpurea* and different amphimictic species (NYGREN, 1951 a).

All these data confirm MÜNTZING's suggestion that »apomixis in *Poa* is due to a rather delicate balance» (1940, p. 171) at the same time as they prove DARLINGTON's interpretation of apomixis as a »blind alley of evolution» (1939, p. 113), as being incorrect. Thus, if the breeder raises a great number of plants in the sexual generations, he will have good chances to select plants, from which later on apomictic generations can be raised. In some cases difficulties will appear to have practically valuable types apomictic enough. The best help in such circumstances is to cross the valuable, segregating type with another form with a strong tendency to aposporous formation of seed. There is a good chance to obtain non-segregating offspring in later generations from such a cross.

From what has been said above the way of breeding *Poa pratensis* seems to be simple. Even in this breeding work there are, however, difficulties which are caused by the apomictic way of reproduction in the species. If any strain of *pratensis* could be used as one parent in a cross to obtain a wanted hybrid combination, this combination would no doubt be easy to produce. The breeder's task is, however, generally to improve the best already existing one, and because of that it is natural to use a strain with a great number of good qualities as a starting material in a cross.

Now, it is a fact that the best *Poa pratensis* strains also are the most constant ones as a consequence of earlier breeding work. Embryologically this means that they do not only have a strong tendency to aposporous E. S. formation, but also that the egg cell in these aposporous sacs divides in such an early stage that a fertilization is more or less excluded. This makes the breeding work very difficult, if such a good strain is wanted as mother in the combination. If the first division in the egg cell has a later starting point in the other species in the combination, it can be made with that species as mother. This method may sometimes have its disadvantages, however. If, for instance, a good *pratensis* strain ought to be improved as to some special qualities like the speed of development, the resistance against some fungi, *etc.*, the breeder's task is to transmit genes for these qualities from the other

Poa species without degenerating the good *pratensis* strain itself. Both species are supposed to be aposporous. If we call the chromosomes of the *pratensis* strain $2P$ and those of the other species $2X$, we will obtain a hybrid with the chromosome complement of $2P+X$, when *pratensis* is used as mother in the combination, while the reciprocal cross gives $2X+P$.

From what has been mentioned above it is obvious that the wanted combination is the first one. In order to estimate the chances to obtain a special hybrid combination an embryological investigation ought to be made of the planned mother strain. This investigation should be worked out in such a way that the relation between the number of sexual and aposporous E. S's is obtained as well as the relation between the E. S's with an early and a late division of the egg cell. When these relations are figured, the possibilities to make the wanted combination can be estimated. If the investigation gives negative results, possibly occurring aberrants in the strain ought to be examined. Such plants often show another type of embryological development than the main bulk of the strain. If there are no aberrants, the strain can be planted out in other places with deviating climates. Aberrants may be found in some of these places.

As an example of the procedure mentioned above may serve a study, which the present writer made at Stanford of one of the *Poa pratensis* strains, which are used in the experiments made at the Carnegie Institution of Washington (NYGREN, 1951 b, p. 114). The strain descended from Mather in the Sierra Nevada at 1.500 *m. s. m.* and was shown by an embryological investigation to form on an average 62,5 % aposporous E. S's and 9,4 % sexual sacs. In the remaining 28,1 % of the cases aposporous as well as sexual sacs were developed, which means either that they both could be completed simultaneously or that one of the sacs would prevail over the other one. As to the offspring, this means that there was reason to suspect that at least 62,5 % of the next generation plants would be of the mother's type, while on the other hand up to 20 % of these plants could deviate from the mother. Some of these deviating plants would be hard to spot, however, because of great morphological similarities to the mother plant. Offspring from the investigated plant had been put out at Stanford in 1945 and in 1946 and at Pullman, Washington, in 1948. The number of aberrants observed in the plantings are to be found in Table 1.

In this case close to four times as many aberrants occurred in the plantings at Pullman compared to the average number at Stanford.

TABLE 1. *Progeny tests of a single individual, 4253—4, of Poa pratensis from Mather.*

No.	Year	Place	No. of plants	No. of aberrants	% aberrants
4439	1945	Stanford	30	2	6,7
4536	1945	»	220	5	2,2
4538	1945	»	251	20	8,5
4758	1946	»	60	0	0,0
In all		Stanford	561	27	4,8
4439	1948	Pullman	83	15	18,1

The figure obtained at Pullman, 18,1 %, fits well with the theoretically estimated one, 20 %. It is a matter of fact that it up to now has not been possible to make a single hybrid with *Poa pratensis* as mother at Stanford, which probably depends upon that the place is not suited for this kind of crossings. In this connection it ought to be mentioned that ÅKERBERG already in 1936 reported the successful production of a hybrid between *Poa pratensis* and *alpina* with the former species as mother (1936 b).

When a wanted hybrid combination has been obtained, the breeding work should be performed in a correct way. If the hybrid segregates, each offspring plant can be divided, and the clone plants planted in different transplant stations, where the individual testing can be made. This is the method used since decades at Stanford. It is also possible to use constant rooms for the primary test. In an experiment worked out at the Earhart Laboratory of California Institute of Technology in Pasadena WILLIAM HIESEY treated the West American *Poa ampla*, a mountain ecotype of *Poa pratensis* from Abisko in northern Sweden, and their hybrid (HIESEY, 1951, p. 102). At a day temperature of 23° C *ampla* did best at a night temperature of 6°, while it at 17° night temperature showed considerably decreased growth and refused to flower. The *pratensis* ecotype, which was adapted to an arctic mountain climate, showed an inverted series, it grew best at 17° night temperature, which could not be expected in advance. The hybrid showed greater tolerance than both parents and did just as well at 6°, 10°, as at 17° night temperature. The experiments lasted half a year, and therefore the results can not depend upon mere chance.

These results can be applied to the practical breeding work, if constant rooms are at hand at the institute where the experiments are made. Such constant rooms should be adjusted to the climates in the planned areas of cultivation. The different seedlings in a segregating

hybrid offspring should be divided, and the clone plants treated in these constant rooms. In this way it will be possible to decide, in which places a propagation of different plants ought to be made, and where a sufficiently variable material will be at hand to give the breeder good chances for a selection of the best types for cultivation.

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ENDOSPERM FORMATION AFTER 2x, 4x CROSSES IN CERTAIN CEREALS, ESPECIALLY IN HORDEUM VULGARE

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IN the late twenties and early thirties the cause of the abortion of seeds formed after species crosses was much discussed. Related species or forms having a different number of genomes give, when crossed, very bad seeds. WATKINS, THOMPSON, THOMPSON and CAMERON, and WAKAKUWA published papers on seed development after reciprocal crosses between tetraploid and hexaploid species of *Triticum*; later, BOYES and THOMPSON included diploid *Triticum* species in the crosses. Hybridizations between diploid, tetraploid and hexaploid species of *Avena* were studied by KIHARA and NISHIYAMA who found that, as in *Triticum*, endosperm development had a different aspect in reciprocal crosses. As a rule, seed development is better when the mother plant has the higher chromosome number; on the other hand, a larger difference in the number of genomes between the crossed plants causes more pronounced disturbances.

The happy idea was conceived by MÜNTZING (1930) that the causes of seed degeneration in the crosses referred to were of a quantitative rather than a qualitative nature. He pointed to the fact that crosses between a diploid and its autotetraploid also result in bad seeds. In normal seeds the relation between the chromosome numbers of the maternal tissue of the ovule, the endosperm and the embryo is 2: 3: 2, but after crosses between plants with different numbers of genomes this ratio in the seeds is altered with physiological and morphological disturbances as a consequence. In a second paper MÜNTZING (1933) more closely discussed this theory and refuted certain other interpretations of the causes of the seed abortion. He pointed out the far-reaching consequence of this kind of sterility facilitating the forming of polyploid species in angiosperms.

Later, seed development in reciprocal crosses between a diploid and its autotetraploid has been investigated, *e. g.*, in the cereals *Secale cereale* (HÅKANSSON and ELLERSTRÖM, 1950; LÖWENSTEIN, 1951) and

Zea mays (COOPER, 1951). In these as in other $2x$, $4x$ crosses — I have studied *Galeopsis pubescens* from the cultures of Professor MÜNTZING (HÅKANSSON, 1952) — development in $2x \times 4x$ and $4x \times 2x$ seeds started normally, but sooner or later the rapidity of the growth of endosperm and embryo slowed down, the endosperm and, later, the embryo finally degenerating. As regards the question of the importance of changed numerical relations between the chromosome numbers of the different parts of the seeds, statements were made by COOPER and BRINK, who investigated *Lycopersicon pimpinellifolium*, HÅKANSSON and ELLERSTRÖM, and HÅKANSSON: the changed relations between the maternal tissue and the endosperm were considered to be the initial cause of the irregular development and degeneration of the endosperm; the death of the embryo was secondary to the disturbed endosperm development.

A favourable material for studying the influence of quantitative changes in such investigations ought to be *Hordeum vulgare*, being autogamous. Professor MÜNTZING and his co-workers have produced tetraploid strains of this cereal, and reciprocal crosses were made between the tetraploids and their diploid parent strains. I have compared seed development after $2x \times 2x$, $2x \times 4x$, $4x \times 2x$, and $4x \times 4x$ crosses; fixations from 10 hours to 21 days after pollination have been investigated. Here only some main results will be reported.

A review of the literature on the cytology and genetics of barley has recently been published (SMITH, 1952). As POPE has shown, in *H. vulgare* fertilization occurs already 20 min. after pollination at the most favourable temperature, 30°—35° C. There are no larger differences between the $2x \times 2x$, $2x \times 4x$, $4x \times 2x$, and $4x \times 4x$ seeds the first days after pollination. After 4 days the development is well on the way, large parts of the endosperm often showing simultaneous divisions.

Endosperm development is rather rapid in barley; after 4 days it has attained the stage that rye endosperm has reached after 6 days. The ovules have now considerably increased in size. It is chiefly the apical part of the ovule which has grown; at the same time the embryosac with endosperm has grown in the apical direction. The antipodals are no longer, therefore, at the end of the embryosac but in an intermediate position. $4x \times 2x$ seeds, as a rule, differ from the other seeds, this apical growth of the ovule and particularly the endosperm being much less pronounced; the endosperm thus formed is short in this cross.

The narrow part of the endosperm near the embryo is filled by tissue after 4 days; cell-formation has often occurred in other larger or smaller parts of the endosperm. The central vacuole is never filled by endosperm. Mitotic disturbances are rare. $2x \times 2x$ seeds perhaps have on the average the largest number of endosperm cells or nuclei. The endosperm in $2x \times 4x$ seeds is well developed and seems to have a number of nuclei comparable with that in $2x \times 2x$. The rarity of mitotic disturbances in $2x \times 4x$ endosperm at this stage of development is striking. In $2x \times 4x$ rye seeds, formation of giant nuclei 4 and 6 days after pollination was very frequent and wall-formation in the endosperm was belated or failed. Barley in these respects is different from rye: only rarely one observes in $2x \times 4x$ a larger degenerating cytoplasm with many nuclei but lacking walls, only in exceptional ovules are irregularities frequent. Such an exception was an ovule with the whole apical part of the endosperm filled by nuclei with doubled chromosome number or telophase pairs with several chromosome bridges. $4x \times 4x$ endosperms are large with a high number of nuclei and well advanced wall-formation. Considerably smaller is, as already indicated, the endosperm in $4x \times 2x$ seeds but wall-formation is very advanced here: the endosperm is often almost completely cellular, the central vacuole, of course, still not filled but surrounded by large, elongated cells, open towards the interior of the vacuole. The endosperm cells in $4x \times 2x$ are large but comparatively few in number, the cells of the outermost layer being smaller.

A retardation of embryo development is not evident in the $2x \times 4x$ or $4x \times 2x$ crosses 4 days after fertilization. In 1951 the embryo had a size of 28—36 cells in good kernels of all the four crosses.

The development 4—10 days after pollination was not closely followed, the first larger fixations being made 10 days after pollination. In *diploid barley* THOMPSON and JOHNSTONE found the central vacuole in the ovule completely filled by endosperm at 6 days, while the first starch appeared in the endosperm at 7 days. In our 10-day-fixations of $2x \times 2x$ seeds the largest part of the endosperm consisted of large cells filled by starch. However, two or three layers at the periphery of the endosperm had a meristematic character; the cells were small, cytoplasmatic and had no starch, mitotic figures were very frequent here. Through the activity of the subepidermal layers where the mitotic figures are directed at right angles to the surface of the endosperm this tissue increases in volume, while the divisions in the epidermic endosperm layer increase the number of cells of this layer. Other re-

gions of the endosperm still lacking starch were the basal part in the vicinity of the embryo and a broad strip along the raphe. This strip consisted of rows of endosperm cells converging towards the raphe and seemed to be a conducting tissue of the nutrients streaming to the endosperm. The differentiation of the embryo, which after THOMPSON and JOHNSTONE begins at the 7th day, may be rather advanced at 10 days.

The development in $4x \times 4x$ *seeds* was studied more superficially, larger differences from the diploid not being expected. A peculiar feature is the occurrence of ovules with very retarded development: several 10-day-ovules had only a few endosperm nuclei; one ovule had a dividing egg-cell and four endosperm nuclei; another ovule had a small embryo of 21 cells and two polar nuclei; no endosperm had been formed. Such slow kernels are small. About the same size have certain somewhat enlarged ovules with egg-apparatus and polar nuclei visible after 10 days, while most unfertilized ovules grow only insignificantly.

In $2x \times 4x$ *seeds* the central vacuole after 6 days was not completely filled by endosperm; this is an indication of somewhat slower development than in $2x \times 2x$. After 10 days the vacuole was filled; the endosperm tissue formed was large but more narrow than normal. A conspicuous feature of seed development in $2x \times 4x$ is the late appearance of starch: at 10 days there is usually no trace of starch in the endosperm, though there may be in the fruit wall. A second feature is that a normal differentiation within the endosperm does not occur, mitosis not being restricted to the peripheral layers, but scattered through the tissue. However, the endosperm tissue is not uniform. It is on the whole poor in cytoplasm but many parts consist of cytoplasmatic cells, which often divide. On the other hand, large regions of the endosperm may consist of cells, having shrunken and no doubt degenerating nuclei. The regions with meristematic activity are in the basal part near the embryo, but may also be in the interior and periphery of the endosperm, and there is similarity to the conditions in $2x \times 4x$ endosperm of *Zea mays* (COOPER, 1951). Giant nuclei indicating disturbed mitosis are now observed in most $2x \times 4x$ endosperms: their number is, however, not large. The embryo is smaller than in $2x \times 2x$ but shows a certain differentiation, the vegetation point being always naked, however.

After 14 days the endosperm tissue is rather large, but in many cases still does not contain any starch. If starch was present, it was only seen in rather small parts of the endosperm. Giant nuclei are now

more frequent. Already after 10 days the endosperm tissue showed a certain looseness, which is now more pronounced; the contact between the individual cells had been weakening and the cells are now often isolated. A meristematic activity of larger parts of the tissue does not occur now, but scattered through the endosperm are small islands of cytoplasm-rich cells often showing mitosis. The islands consist of one to a few cells. The embryo was often small and starved, but sometimes larger and irregular, with no primary root or enclosed organized plumula. One kernel was exceptional, having the main part of the endosperm filled with starch; corresponding to the more normal endosperm was a highly differentiated, normal embryo. This embryo had about 21 chromosomes and the kernel was, therefore, not the result of an experimental error. — After 21 days the endosperm was dissolving, the small islands floating among the debris. All observed embryos were abnormal, but also more normal ones must occur.

The development of the $4x \times 2x$ *seeds* is very different. After 6 days the endosperm was considerably smaller than in $2x \times 4x$, but it had attained a more advanced stage, the central vacuole being completely filled by tissue. After 10 days the $4x \times 2x$ endosperms contain much starch, but its distribution within the tissue is not the usual one. Above the embryo are large masses, while neighbouring parts of the endosperm may lack starch. The volume of the endosperm in the best kernels is also considerably smaller than in $2x \times 2x$ or $4x \times 4x$; the outer layers show no or few mitosis figures, this also indicating an arrested growth of the endosperm. The basal part of the endosperm is well developed, while its apical part is reduced. The embryo showed reduced size, though it is more advanced concerning its morphological differentiation than the embryo of $2x \times 4x$ seeds; the vegetation point of the embryo was enclosed in the coleoptile tissue. — Many 10-day-seeds were very small, containing a very small endosperm tissue. The outermost layer of this endosperm consisted of cells rich in cytoplasm; the inner tissue consisted of vacuolate cells of irregular form and with shrunken nuclei but containing starch. A further development of such seeds does not occur: in other similar small seeds the inner endosperm tissue had degenerated. One also may find seeds, as in $4x \times 4x$, with very belated development having only a few free endosperm nuclei; in one seed the egg-nucleus was dividing. Such belated seeds were not observed in $2x \times 2x$ and $2x \times 4x$ and therefore seem to be due to the tetraploid condition of the mother plant.

After 14 and 21 days the kernels were in a bad condition. The

endosperms were small, and the embryo had undergone only an insignificant further development. The embryo cells were poor in content and had no starch, the embryo being thus starved. Probably better seeds are sometimes formed than could be investigated here.

CONCLUSIONS.

In conformity with previous findings in rye and maize initial development in $2x \times 4x$ and $4x \times 2x$ seeds in barley is good, disturbances setting in later. These disturbances have a different aspect in the reciprocal crosses though the final result, degeneration of endosperm and embryo, is the same. The $2x \times 4x$ endosperm of barley attains a large size, but the storage of starch in the endosperm is very belated, and occurs only within a small part of the tissue. A peculiar feature is the prolonged, though locally limited, mitotic activity in the endosperm causing outgrowths from periphery and ingrowth, towards the interior of the endosperm. The $4x \times 2x$ endosperm is small, and starch is stored very early. This endosperm becomes cellular more early than normal, showing a weak mitotic activity which stops early. The embryo also is different in the two crosses, as a rule being abnormal and not forming an enclosed plumula and primary root in $2x \times 4x$.

These peculiarities of $2x \times 4x$ and $4x \times 2x$ seeds are in several respects of opposite tendency and a comparison with observations made in maize (by COOPER) and in rye shows several similarities between these cereals, though also certain differences. In $2x \times 4x$ seeds the following peculiarities of the endosperm may be emphasized. (1) Mitotic irregularities, especially the forming of giant nuclei, are common in the endosperm after many low-chromosome × high-chromosome species crosses (instances are given in HÅKANSSON and ELLERSTRÖM). In $2x \times 4x$ rye irregularities are frequent after 4 and 6 days, but in maize they appear later, after 16—24 days; barley, in this respect, is more similar to maize. The early appearance of irregularities in rye may be connected with the fact that disturbances are not uncommon in the endosperm of ordinary, diploid rye. On the other hand, in barley endosperm, which has been formed after crosses with rye, mitotic irregularities are very pronounced, and appear very early, sometimes at the division of the polar nuclei. BRINK and COOPER are of the opinion that the rye sperm has failed to stimulate the antipodals to normal activity, and therefore these cells cannot regulate the endosperm divisions in a normal way. The hypothesis of the importance of the anti-

podals in this respect was, however, not accepted by THOMPSON and JOHNSTONE. (2) Belated formation of cells in the nuclear endosperm has been reported in many low-chromosome × high-chromosome crosses; in $2x \times 4x$ rye this delay was very conspicuous (HÅKANSSON and ELLERSTRÖM). In maize, however, cell formation seems only slightly belated and the same holds good for barley. (3) Abnormal meristematic activity was shown by the endosperm tissue in barley and also in maize where the growth of the basal part of the endosperm resulted in an enclosement of the embryo in endosperm tissue. In $2x \times 4x$ rye this irregular growth was not observed. (4) Starch is deposited very late and usually in small quantities in $2x \times 4x$ endosperm of rye and barley; this is also valid for maize, as is clear from the description of this endosperm by COOPER.

$4x \times 2x$ endosperms have, on the other hand, the following properties. (1) Mitotic irregularities are rare. (2) Cell formation is early. In rye it was evident hat tetraploid rye formed endosperm cells earlier than diploid rye: in $4x \times 2x$ cell formation was still earlier. In $4x$ barley it was doubtful if there were any larger difference in this respect between $4x \times 2x$ and $4x \times 4x$, but it was earlier than in diploid barley. COOPER states that the endosperm in $4x \times 2x$ maize becomes cellular at an early stage. (3) Prolonged abnormal meristematic activity does not occur. The volume of the endosperm is small and mitosis stops early. (4) Deposition of starch begins early. This has been observed in rye and barley and also in maize: in the endosperm of diploid maize starch appears after 12 days; in $4x \times 2x$ endosperm after 6 days (COOPER).

In $2x \times 4x$ seeds of maize and barley the embryo is very often irregular, a normal plumula and primary root not being differentiated. In the $4x \times 2x$ crosses embryo development is very retarded, and the embryo is starved. Only rarely germinable seeds are formed. The best fertility shows maize where 4,2 % of the $4x \times 2x$, and 0,3 % of the $2x \times 4x$ seeds were germinable. In barley only two 20-chromosome plants have been obtained in $4x \times 2x$ (oral communication of Professor MÜNTZING and Dr. TOMETORP). The rye crosses seem to show somewhat better fertility, judging from published data.

The genic constitution of the barley endosperm is, disregarding the different number of genomes, the same in $2x \times 4x$ and $4x \times 2x$. The different aspect of the seed abortion in the reciprocal crosses may, as has been pointed out long ago, be due to the fact that the quantitative relations within the seeds are different. Another cause of the

reciprocal differences are the different morphological and physiological constitution of the mother plant, the tetraploid showing certain peculiarities compared with the diploid, which reappear, sometimes underlined, in the $4x \times 2x$ seeds.

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ÜBER DIE ENTSTEHUNG DER SELBSTFERTILITÄT BEIM ROGGEN

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MEINE Versuche mit den Fertilitätsverhältnissen des Roggens dauerten während der Jahre 1912—1927. Seit 1916 habe ich nichts davon berichten können, weil die Anhäufung des Materiales, meine Beschäftigung mit dem Inzuchtsproblem (HERIBERT NILSSON, 1937), sowie meine artbildungstheoretische Forschung meine ganze müssige Zeit genommen haben. Hier möchte ich nur einen sehr geringen Teil meines Materiales behandeln, nämlich das Entstehen der Selbstfertilität aus den selbststerilen Roggensorten. Dazu knüpfe ich nur einige Bemerkungen zu dem Vererbungsgang der Selbstfertilität an. Über meine sehr umfassenden Isolierungsversuche zu dieser Frage hoffe ich bald berichten zu können, weil die Resultate schon tabellarisch bearbeitet vorliegen.

In meiner Übersicht der ersten vier Versuchsjahre (HERIBERT NILSSON, 1916) habe ich vermutet, dass die ausgeprägte Selbstfertilität, die Hochfertilität, eine rezessive Eigenschaft des Roggens sei. Ich hatte dann nicht die Kreuzung hochfertil × selbststeril ausgeführt, sondern schloss diese Annahme daraus, dass meine sämtlichen hochfertilen Pflanzen aus selbststerilen hervorgegangen waren. Und sie waren sogleich konstant. Das weist ausgesprochen auf Rezessivität hin.

Indessen war es auch auffallend, dass die hochfertilen Pflanzen erst *nach Isolierung* hervortraten. Von den *direkt* isolierten Pflanzen aus einer freien Population zeigte keine einzige eine bemerkenswerte gesteigerte Fertilität.

In dem Bericht 1916 habe ich diese Pflanzen als I_1 bezeichnet. Richtiger ist es sie als I_0 und erst die erste *Nachkommenschaft* einer Isolierung als I_1 zu bezeichnen. I_0 ist also eine Pflanze, wo der Fertilitätsprozent wohl durch Isolierung ermittelt ist, aber die Pflanze selbst hat keine Isolierung durchgemacht.

Man kann natürlich vermuten, dass die gefundene ausgesprochene Selbststerilität jedoch keine allgemeine Eigenschaft des Roggens ist, sondern dass ein grösseres Material und dazu aus verschiedenen Sorten und Züchtungspopulationen andere Resultate geben kann.

TABELLE 1. *Isolierungen von 117 Pflanzen des Petkuser Roggens (I_0) und ihrer Nachkommenschaften (I_1).*

I_0				I_1			
Feld-nummer	Nr. der Pflanze	Anzahl isolierter Ähren	Fertilitätsprozent	Feld-nummer	Nr. der Pflanze	Anzahl isolierter Ähren	Fertilitätsprozent
443—25	a	2	0,8				
	b	10	3,1	281—26	b	1	2,1
					c	1	3,4
					d	7	0,7
					e	6	1,5
	c	8	1,0	282—26	a	1	0
					b	5	0,3
					c	5	0
	d	8	0,7	283—26	a	5	1,9
	e	6	1,3	284—26	a	2	0,8
					b	1	0
	f	7	0,3	285—26	a	5	0
	g	7	3,1				
	h	7	0				
	i	6	0				
	j	6	0,2				
	k	12	1,9	288—26	a	5	0
					b	4	1,3
	l	12	1,5	289—26	a	1	0
	m	13	1,3	290—26	a	5	0,3
					b	5	0
	n	10	1,6	291—26	a	3	0
					b	2	0
					c	5	0,8
					d	3	0
	o	10	1,7	292—26	a	5	0,5
	p	10	0,7	293—26	a	5	51,6
	q	10	0,5	294—26	a	5	10,9
					b	4	0
	r	10	1,0				
	s	10	0,2				
	t	10	1,1	297—26	a	5	0
	u	9	0,2	298—26	b	3	1,1
					a	5	0,3
	v	9	7,4	299—26	a	5	2,3

I_0				I_1			
Feld-nummer	Nr. der Pflanze	Anzahl isolierter Ähren	Fertilitätsprozent	Feld-nummer	Nr. der Pflanze	Anzahl isolierter Ähren	Fertilitätsprozent
	b	4	0				
	c	5	1,9				
449—25	a	5	0,6				
	b	5	2,3	319—26	a	5	0
	c	5	0				
450—25	a	5	0				
	b	5	0				
	c	5	0				
451—25	a	5	2,3				
	b	5	0				
	c	5	0				
452—25	a	5	0				
	b	5	0,3				
	c	5	0				
453—25	a	4	0,8				
	b	5	7,2				
	c	5	0,3				
454—25	a	5	0				
	b	4	1,3	320—26	a	3	0
					b	2	0
					c	3	0
	c	5	0				
455—25	a	3	0,4				
	b	4	0,7				
	c	5	0				
456—25	a	6	0,8				
	b	5	1,8				
	c	5	1,4				
457—25	a	5	0,3				
	b	5	1,1				
	c	4	1,2				
458—25	a	9	0				
	b	6	0,4				
	c	3	0				
459—26	a	5	0				
	b	5	0,3				

	u	9	0,2	298—26	b	3	1,1			b	6	0,4							
	v	9	7,4	299—26	a	5	0,2			c	3	0							
					b	5	2,2		459—26	a	5	0							
					c	5	1,9			b	5	0,2							
	x	8	2,1	300—26	a	5	1,2			c	5	0,2							
					b	5	2,2		460—26	a	5	2,0							
	y	8	1,2	301—26	a	4	0,6			b	5	0,2							
					b	5	0			c	5	0,8							
	z	8	0,4	302—26	a	3	0		461—26	a	5	0,2							
	å	7	0							b	4	0,8							
	ä	7	0,2	303—26	a	6	0			c	5	4,4	325—26	a	1	4,6			
	ö	7	0,5	304—26	a	4	51,9							b	1	2,4			
	aa	6	2,0	305—26	a	3	0,5							c	5	0,8			
					b	4	0,6							d	7	0			
					c	3	0		462—26	a	5	0,7		e	6	0,8			
					d	3	1,2			b	5	0							
					e	3	1,1			c	4	0							
					f	1	0		463—26	a	4	0,8							
	bb	6	0,9	306—26	a	5	1,4			b	5	0,2							
	cc	6	0,2	307—26	a	4	0			c	5	1,2							
	dd	6	2,2	308—26	a	3	0		464—26	a	5	0,4							
					b	2	84,0			b	5	0,6							
	ee	5	0,6	309—26	a	4	0			c	5	0							
					b	3	41,5		465—26	a	5	0,9							
	ff	5	0,4							b	5	0,7							
	gg	3	6,5	311—26	a	5	0			c	5	0,6							
					b	4	0		466—26	a	10	0,9							
444—25	a	5	0,7							b	7	0,6							
	b	5	1,4							c	5	0,3							
	c	5	1,1						467—26	a	5	1,6							
445—25	a	5	0,7							b	5	1,5	329—26	a	2	1,3			
	b	5	2,7							c	5	0							
	c	5	0,6						468—26	a	18	0,2							
446—25	a	5	1,3							b	5	0,3							
	b	5	0,6							c	4	0,9							
	c	5	0,3						1192—26	a	6	0,3							
447—25	a	5	2,1	315—26	a	4	64,4			b	6	0,6							
	b	4	1,7		b	5	0			c	6	1,8							
					c	5	0			d	6	1,9							
					d	4	47,0			e	5	0,3							
	c	5	0,3	317—26	a	5	0,2			f	5	0,3							
448—25	a	5	1,0							g	1	2,8							

Um diesen Verhältnissen näher zu kommen, habe ich zuerst die allbekannte Sorte Petkuser Roggen, von dem die Mehrzahl neuerer Sorten Europas abstammen, ausgewählt. Diese Sorte war auch in meinen erwähnten früheren Versuchen die Hauptsorte.

Tabelle 1 gibt den Prozentsatz der Fertilität für I_0 und I_1 ausführlich wieder. Ich möchte in bezug auf die Isolierungstechnik auch eine Bemerkung beifügen. Alle Isolierungen wurden durch doppelte Pergamintüten ausgeführt. Der Halm wurde weiter bei der Umbindungsstelle der Tüte mit Baumwolle umwickelt, sodass jede Möglichkeit eines Eindringens von Pollen ausgeschlossen war. Die Versuche sind also unter *ganz* sicheren Kautelen durchgeführt worden, was bei Untersuchungen über Pollinationsverhältnisse der Windbestäuber eine erste und absolute Forderung ist (vgl. HERIBERT NILSSON, 1917).

Es ist sogleich bei der Betrachtung der Tabelle 1 ersichtlich, dass *nur* sehr niedrige Zahlen der Fertilität auftreten. Unter 117 Pflanzen haben nur 2 (7,2 und 7,4 %) einen höheren Fertilitätsprozent als 5 %; 22 (18,8 %) sind ganz selbststeril und 78 (66,7 %) liegen unter dem Prozentsatz 1 %. Keine einzige Pflanze erreicht den Fertilitätsgrad 10 %. Der Petkuser Roggen ist deshalb eine typisch und ausgesprochen selbststerile oder sehr niedrig fertile Roggensorte.

Dass die *total* selbststerilen Pflanzen eine besondere Gruppe in bezug auf die befruchtungsbiologischen oder genischen Verhältnisse repräsentieren sollten, ist nicht wahrscheinlich. Die Grenze ist von 0—1 % und weiter aufwärts ganz fliessend. Weiter gibt eine Pflanze mit sehr niedrigem Prozentsatz eine Nachkommenschaft mit erhöhtem (0,9 gibt 1,4; 0,7—1,9; 0,5—10,9) oder eine mit erhöhtem Prozentsatz ganz selbststerile Pflanzen (1,2—0,0; 2,3—0,0; 6,5—0,0). Man hat offenbar eine modifikativ variable Selbststerilität, keine totale. Im folgenden verwende ich deshalb den Ausdruck »selbststeril» für einen sehr niedrigen Körneransatz, unter 10 %. Die Grenze ist natürlich willkürlich, hat aber in dem *Resultat* des Experimentes eine Motivierung, indem *keine einzige Pflanze* von *höherem Fertilitätsgrad als 10 %* auftritt.

Aus Petkuser Roggen stammen die schwedischen Sorten Stern Roggen von Svalöf und meine Züchtung Sturm Roggen II. Die erste ist aus einer Züchtungsmethode, die modifizierte Familienzüchtung genannt werden kann, die letztere aus Züchtung durch Separation, schon betreffs der Nachkommenschaft der Elitenpflanze, hervorgegangen. Sturm Roggen II bezeichnet ausserdem die zweite Auslese (E_2). Stern Roggen ist deshalb eine schwache Einengung der Variabilität des Petkuser Roggens, Sturm Roggen II eine bedeutend effektivere. Falls wirk-

lich hochgradig selbstfertile Pflanzen in der Petkuser-Population zu finden wären, würde man sie vor allem in dem verminderten Variabilitätskreis des Sturm Roggens antreffen.

In Tabelle 2 habe ich das Resultat der Isolierungen der erwähnten Sorten mitgeteilt, sowie auch das einer zweiten E_2-Nachkommenschaft, Nr. 644. Da es weiter von grösster Wichtigkeit ist, auch ein Roggenmaterial, das in bezug auf die Variabilität weit verschieden von dem Petkuser Roggen ist, zu prüfen, war es mir eine grosse Freude, dass ich von dem hervorragenden, so bedauerlich verschiedenen russischen Pioniärforscher VAVILOV während der Jahre 1923—1926 mehrere russische Roggensorten sowie auch Proben seiner berühmten Unkrautroggen von S. W. Asien erhalten und prüfen konnte. Zusammenfassende Übersichten sämtlicher dieser Versuche findet man in Tabelle 2, die mit einer Zusammenstellung der Tabelle 1 beginnt.

In der Tabelle sind nicht nur die direkt aus den Sorten isolierten Pflanzen (I_0), sondern auch ihre Nachkommenschaften (I_1) aufgenommen. Auf die Verteilung der Varianten der letzten Generation komme ich unten zurück.

Betrachten wir nun die Verteilung der Varianten in I_0, so finden wir, dass diese *ganz dieselbe* ist, wie wir sie schon für Petkuser Roggen gefunden haben. Alle Pflanzen, ganz unabhängig davon, ob sie eingeengte Populationen von dieser Roggensorte oder ob sie wildwachsende, in bezug auf die Variabilität sehr erweiterte Populationen sind, sind *alle ausgesprochen selbststeril.* Die Variabilitätskurve ist etwas erweitert, was natürlich ist, da nun die Individuenzahl 443 statt 117 ist. In der Klasse 10—20 % fallen 13 Individuen, d. h. 3 %, und zwischen 20—30 % liegt ein einziges Individuum (siehe Tabelle 2, links). *Hochfertile Pflanzen fehlen ganz.*

BREWBAKER (1926) hat umfassende Isolierungen während der Jahre 1920—1925 durchgeführt. Seine Pflanzen wurden aus Michigan Roggen, welche Sorte schwedischer Abstammung ist, ausgewählt. Seine Resultate sind nicht für die einzelnen Pflanzen angegeben, sondern als Durchschnittsertrag einer Nr.-Gruppe, von welcher die Anzahl isolierter Pflanzen angegeben wird (1—7). Eine Umrechnung seiner Versuchsresultate für das Jahr 1925, in dem er die grösste Anzahl I_0-Isolierungen hat, nach Fertilitätsklassen, mit Angabe auch des Mediums der Klasse, ergibt folgendes Resultat (Tabelle 3).

Ganz wie in meinen Versuchen liegt also die Mehrzahl der Pflanzen unter dem Fertilitätsprozent 5 % und fast alle unter 10 %. Hochfertile Pflanzen kommen auch hier nicht vor.

TABELLE 2. *I_0- und I_1-Isolierungen verschiedener Sorten und Unkrautpopulationen von Roggen.*

Sorte	Anzahl der Pflanzen	*I*-Generation	Fertilitätsprozent										
			0	0—5	5—10	10—20	20—30	30—40	40—50	50—60	60—70	70—80	80—90
Petkuser R.	117	I_0	22	93	2								
	66	I_1	29	30	—	1	—	—	2	2	1	—	1
Stern R.	40	I_0	3	34	2	1							
	39	I_1	11	12	2	8	2	1	—	1	1	1	—
Sturm II R.	59	I_0	11	46	1	1							
	59	I_1	12	28	1	5	1	5	3	3	—	—	1
Nr. 644 aus Petkuser	110	I_0	18	83	6	3							
	153	I_1	39	71	13	12	4	2	7	2	2	1	—
Persischer R.	14	I_0	1	10	2	1							
	25	I_1	11	8	—	3	1	2	—	—	—	—	—
Pamir R.	13	I_0	2	9	—	2							
	4	I_1	2	1	—	—	—	—	—	1	—	—	—
Russischer Landroggen	49	I_0	9	29	7	3	1						
Kaukasischer R.	13	I_0	3	8	2								
Unkrautroggen aus Turkestan	20	I_0	6	13	1								
Unkrautroggen aus Afghanistan	8	I_0	1	3	2	2							
Summe I_0	443		76	328	25	13	1						
Summe I_1	346		104	150	16	29	8	10	12	9	4	2	2

TABELLE 3. *Fertilität der I_0 von 100 Pflanzen aus Michigan Roggen in den Versuchen 1925 von* BREWBAKER *(1926).*

Fertilitätsprozent					
0–5 %		5–10 %		> 10 %	
Anzahl der Pflanzen	M	Anzahl der Pflanzen	M	Anzahl der Pflanzen	M
73	2,0 %	23	6,5 %	4	13,1 %

LUNDQVIST (1947) hat über die Fertilität von tetraploidem Roggen der Sorte Stahl Roggen, die aus Petkuser abstammt, berichtet. Von 268 isolierten Pflanzen der I_0 hatten 223 einen Ansatz unter 5 % und 40 unter 20 %. Nur 5 Pflanzen hatten eine höhere Fertilität, lagen zwischen 20—35 %. Hochfertile Pflanzen kamen also auch in der tetraploiden I_0-Isolierung nicht vor.

Ein vollkommen neues Bild der Fertilitätsverhältnisse des Roggens erhalten wir, wenn wir die *I_1-Nachkommenschaften* der Tabelle 2 betrachten. In *jeder* Sorte, ganz unabhängig davon, ob diese eine *Kultursorte* oder *Unkrautroggen* ist, treten nun *hochfertile* Pflanzen auf. Ein Blick auf Tabelle 2 zeigt dies sehr auffallend. Die rechte Hälfte der Tabelle, die für I_0 leer ist, zeigt für I_1 auf total 346 Pflanzen in den Klassen 20—90 % nicht minder als 47, d. h. 13 %. Eine Fertilität, die *höher als 50 % ist, haben 17 Pflanzen, d. h. 5 %*. Es ist offenbar, dass bei Isolierung von Roggen *selbstfertile Pflanzen zuerst in der Nachkommenschaft schon isolierter Pflanzen hervorgehen, nicht aber in den frei abgeblühten Populationen unter den selbststerilen gefunden werden können.*

Eine Erklärung dieser ganz sonderbaren Erscheinung könnte vielleicht in den genialen Deutungen der Selbststerilität bei *Nicotiana* von EAST und MANGELSDORF (1925) gesucht werden. Das hat schon PETERSON (1934) vermutet und auch durch Kreuzungsexperimente zu bestätigen versucht. Die genannten Forscher fanden nämlich, dass eine Serie von Allelomorphen diese Eigenschaft bedingen. Kam es vor, dass eine Pflanze, die z. B. S_1S_2 war, mit Pollen von S_1 oder S_2 bestäubt wurde, wuchsen die Pollenschläuche nicht aus. Wurde dagegen S_3- oder S_4-Pollen oder überhaupt Pollen jedes anderen Alleles (sie nehmen 25 solche an) verwendet, so tritt Befruchtung ein. Dieselbe Pflanze, die früher selbststeril war, wird nun fertil.

TABELLE 4. *Isolierung der Nachkommenschaften einer Kreuzung von Nr. 258 (selbststeril)×Sjf. I (selbstfertil) durch 7 Generationen. Die Kreuzung war $I_3 \times I_5$.*

Abstammung	Fertilität der Eltern	Feld-nummer	*F*- (resp.) *I*-Generation	Fertilitätsprozent					
				0	0–5	5–10	10–20	20–50	50-100
52–19×10–19	2,1 resp. 54,7	48—20	F_1 (I_0)						10
48—20, a	54,5	29—21	F_2 (I_1)			1	1	2	16
48—20, b	86,8	30—21	» »					1	23
29—21, a	70,8	17—22	F_3 (I_2)					6	31
29—21, b	50,2	18—22	» »				4	16	1
29—21, c	56,4	19—22	» »				1	13	8
29—21, d	56,9	20—22	» »					4	30
29—21, e	64,9	21—22	» »					13	21
29—21, f	72,4	22—22	» »					7	21
29—21, g	68,1	23—22	» »				1	2	17
29—21, i	67,3	24—22	» »				1	17	11
29—21, j	54,3	25—22	» »				5	11	
29—21, h	8,4	26—22	» »		1	1	1		
30—21, a	72,5	27—22	» »			1		9	9
30—21, b	79,3	28—22	» »			1	1	14	21
30—21, c	78,8	29—22	» »					5	19
30—21, d	73,8	30—22	» »		2	1	6	16	11
30—21, e	84,0	31—22	» »		1		1	17	27
30—21, f	76,4	32—22	» »	1		1		4	26
30—21, g	57,7	33—22	» »		1		1	23	7
30—21, h	78,1	34—22	» »				2	6	23
30—21, i	64,3	35—22	» »					16	23
17—22, a	69,2	67—23	F_4 (I_3)				1	2	
18—22, a	19,7	68—23	» »	1					
19—22, a	59,8	70—23	» »				1	2	
19—22, b	48,5	71—23	» »	1	1		3	3	
20—22, a	40,9	72—23	» »		3	2	4	5	
20—22, b	73,6	73—23	» »					9	
23—22, a	51,0	74—23	» »				4	8	
23—22, b	61,1	75—23	» »			2			
27—22, a	45,0	76—23	» »		2	1	8	3	
27—22, b	60,4	77—23	» »		2	2	3	2	1
32—22, a	55,1	78—23	» »			1	11	2	
32—22, b	73,0	79—23	» »		1	2	5	10	
70—23, a	41,6	329—24	F_5 (I_4)				1		
71—23, a	17,9	330—24	» »				3		
77—23, b	35,5	332—24	» »					1	
78—23, a	19,1	333—24	» »					3	
329—24, b	16,1	260—25	F_6 (I_5)					2	
260—25, a	40,7	133—26	F_7 (I_6)				1	1	
			Summe	3	14	16	70	255	356

Es ist ja deutlich, dass durch diese Annahme die durchgehende Selbststerilität der I_0 erklärt werden kann. Denn der eigene Pollen wird ja verwendet. Nichts neues wird zugeführt, wie bei freiem Abblühen.

Aber kann auch das ganz regelmässige Auftreten hochfertiler Pflanzen in I_1 dadurch erklärt werden? Soweit ich sehen kann, ist dies ausgeschlossen. Neue Allele sind ja durch die fortgesetzte Isolierung definitiv abgesperrt.

Nun haben indessen EAST und YARNELL (1929) ein weiteres Allel dieser multiplen Serie gefunden (S_f), das die prohibitive Zertation aller übrigen Allele aufhebt. Eine S_1S_f-Pflanze wäre deshalb selbstfertil. Aber wenn man annimmt, dass ein solches Gen auch bei dem Roggen zu finden wäre, weshalb tritt dann nicht Selbstfertilität schon in I_0 auf?

Ich erwähnte, dass ich früher die Selbstfertilität, ehe ich noch Kreuzungen ausgeführt hatte, als rezessiv ansah, weil sie immer aus Selbststerilen ausgespalten wurde. Wie erhalten sich diese Eigenschaften dann bei Kreuzung?

Diese Frage kann ich beantworten, wenn ich auch auf die Spaltung in dieser Mitteilung nicht näher eingehe. Eine typische Spaltungsserie möchte ich indessen erwähnen. In Tabelle 4 wird eine Kreuzung Selbststeril $\times$ Selbstfertil durch 7 Generationen demonstriert. Die Eltern hatten den Ansatzprozent von Körnern 2,1, resp. 54,7 %.

Die vollständige Dominanz von hochfertil in F_1 ist deutlich. Alle F_1-Pflanzen (also I_0) sind hochfertil, fallen in der Klasse 50—100. In F_2 und weiteren Generationen spalten sehr wenige Selbststerile aus. Ziehen wir die Grenze bei 10 %, wird das Verhältnis selbstfertil : selbststeril 690 : 33, d. h. 20 : 1. Mindestens zwei polymere Fertilitätsfaktoren mussten an dieser Spaltung beteiligt sein.

Es sieht also so aus, als ob wir auch beim Roggen eine Serie von multiplen Allelen hätten, die eine prohibitive Zertation verursachte, ganz wie sie EAST für *Nicotiana* aufgewiesen hat, und wie diese Erscheinung später für mehrere Gattungen (*Petunia*, *Antirrhinum*, *Oenothera*, *Lythrum*, *Fagopyrum*; Zusammenstellung bei STRAUB, 1948) konstatiert worden ist. Aber erst wenn diese Zertation durch aufgezwungene Selbstbefruchtung so zu sagen »abgeschirmt» ist, tritt das wirkliche genische Spiel anderer Fertilitätsfaktoren auf. Wie nun die Verhältnisse in dieser Hinsicht liegen, darauf kann vielleicht mein weiteres Material etwas mehr Licht werfen. Diese Auseinandersetzung zur Problematik der Fertilitätsbiologie des Roggens hoffe ich bald erstatten zu können.

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A NEW TYPE OF ACHONDROPLASIA IN CATTLE

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SEVERAL types of inherited skeletal abnormalities in cattle are described in the literature. Many of these abnormalities show a rather constant manifestation, whereas others are more variable, *e.g.* the defect »amputated» (JOHANSSON, 1941). On the whole, however, the penetrance of the causative genes seems to be high which facilitates the genetical analysis. Thorough descriptions of the different types of disturbances in the skeletal development of birds and mammals, including their manifestations during fetal as well as post-fetal growth, would seem to be of great interest in relation to the mode of gene action, as suggested by LANDAUER's investigation on the Creeper fowl referred to below.

Achondroplasia, or *chondrodystrophia fœtalis*, characterized by a defective development of the chondrous skeleton, constitutes an important group of skeletal abnormalities. There is a reduction in length of some or all of the skeletal parts of the limbs, producing varying degrees of micromelia, but also the head and in some cases the trunk may be affected. Thus different types of disproportionate dwarfism arise; the »bull-dog» calves are perhaps the best known examples. In a recent review GILMORE (1949) distinguishes between four types of achondroplasia in cattle:

(1) The bull-dog calves of the Dexter breed, described by SELIGMAN (1904), CREW (1924) and others, are usually aborted after six to eight months of pregnancy. These non-viable fetuses look very monstrous; the limbs are extremely short, the cranium bulging, the nose depressed, and the lower jaw protruding; the tail seems to take origin far up on the back, and usually there is a large umbilical hernia. The manifestation of the defect is rather uniform, and is due to homozygosity for a gene which has a marked effect also in the heterozygotes. Characteristic for the Dexter cattle is a short and broad head and short legs, especially below knee and hock. When these Dexters are mated *inter se* they produce bull-dogs, Dexters and animals with legs of normal length, in the proportion 1 : 2 : 1. The viability of the

heterozygotes seems to be quite normal in all respects. — The same abnormality has been found in several other breeds, apparently due to independent mutations at the same gene-locus. MEAD, GREGORY and REGAN (1946) have traced one such mutation in the Jersey breed to a certain bull, who had normal parents but was himself of the »Dexter type».

(2) Another less extreme but also rather uniform type of bull-dog calves was first described by WRIEDT (1925) in the Telemark cattle of Norway. The affected calves are usually born alive after a gestation of normal length; in most cases they die within a few days but one calf lived 14 days. The causative gene is strictly recessive and shows no effect in the heterozygotes. — The same type of bull-dogs has been found in several other breeds, *e. g.* the Swedish Friesians.

(3) A third form of achondroplasia appeared in an inbreeding experiment with Jersey cattle (GREGORY, MEAD and REGAN, 1942). This defect is extremely variable in its expression; both the axial and appendicular skeleton may be affected. All abnormal calves had a short, broad head. The extreme type was not viable, but one heifer with mild achondroplasia lived to 14 months of age until she was slaughtered. The length of legs is little affected, if anything at all. The defect is inherited as an autosomal recessive.

(4) The fourth type is found by LJUTIKOV (1937) in the Jaroslav breed in Russia. The affected animals are characterized by short legs and more or less shortened mandible. This type of achondroplasia has little influence on the viability of the animals, except in cases where the mandible is very short, and the animals, therefore, have difficulties in eating. Here also the inheritance seems to be that of a Mendelian recessive.

LUSH (1930) has reported on »duck-legged», grade Herefords with normal head and body but a considerably shortening of the long bones in the limbs. The same type of short-leggedness has been found also in other breeds. The causative gene is said to be dominant but it is not definitely known whether or not there is any noticeable difference between the homo- and heterozygotes. »Duck-leggedness» may be considered as a fifth type of achondroplasia in cattle, corresponding perhaps to the achondroplastic human dwarfs (MØRCH, 1941). Short-leggedness in the Ancon sheep (CHANG, 1949 a and b) is phenotypically similar but depends on a recessive gene. The growth-rate and viability of the »duck-legged» cattle are approximately normal.

Among other inherited skeletal abnormalities the »amputated»

calves may be mentioned, which were first described by WRIEDT and MOHR (1928), although they should perhaps not be classified as achondroplasts. The degree of »amputation» is very variable. In extreme cases the upper jaw is greatly underdeveloped, and the lower jaw as well as the fore and hind limbs may be almost completely absent, but other cases have been found where the head is almost normal and the legs are »amputated» in the metacarpal and metatarsal regions. This defect, as well as short spine (MOHR and WRIEDT, 1930), missing phalanges (JOHANSSON, 1942), wrytail (ATKESON *et al.*, 1944), and screwtail (KNAPP *et al.*, 1936), is completely recessive to the normal type.

Proportionate dwarfism in Jersey cattle is described by MEAD *et al.* (1942), and seems to be due to a recessive gene. These »dwarfs» are normal at birth but later on growth is retarded; the dwarfishness can not be accurately diagnosed until about 12 months of age. The reproductive efficiency of the dwarfs is lower than normal. The dwarfism seems to be due to an endocrine disturbance manifesting itself gradually during the post-natal development. The same type of dwarfism has been found also in other breeds (BAKER *et al.*, 1951).

The most thoroughly studied case of hereditary achondroplasia in domestic animals would seem to be that of the Creeper fowl, on which LANDAUER has reported in a series of publications. In heterozygotes the principal effect of the mutant gene is a pronounced shortening of the long bones of the legs. In homozygotes, if they survive the lethal period during the early part of incubation, both the head and the extremities are involved. The case is parallel to that of the Dexter bulldog calves. LANDAUER (1941) points out that all available evidence indicates that the peculiarities of the Creeper embryos are preceded by a developmental retardation of the embryo as a whole; limb buds of genetically normal embryos, grown in vitro under growth-retarding culture conditions, showed features typical of homozygous Creeper embryos. LANDAUER assumes, therefore, that general factors act on the embryo as a whole, and that the parts reflect differentially the importance of this disturbance.

ACHONDROPLASTIC CALVES IN THE SWEDISH RED AND WHITE BREED.

In the autumn of 1948 a breeder of Swedish Red and White Cattle reported to the present writer that a considerable number of malformed calves were born after a certain bull in two different herds. The bull, 38 Hanorps Hero, born the 17th of September 1945, was used in one

of the herds from March to November 1947 when he was sold to the other herd where he served until slaughtered in March 1949. The bull himself was completely normal, and none of his relatives were known to have produced any malformed calves. The cows in the first herd were bought from several sources and represented different lines of descent. They were not all pure-bred; one of them belonged to the Swedish Friesian breed. In this herd the bull produced 13 calves, 9 of which showed the same typical malformation of the head and legs, and only 4 were normal. In the other herd the bull produced 40 calves, 16 of which were malformed and 24 normal. Most of the cows in this herd were daughters of two bulls both of which, however, were unrelated or only very distantly related to 38 Hanorps Hero. The total record for the bull is 25 malformed and 28 normal calves. The sex ratio of the malformed calves was normal, *viz.* 13 ♂♂ : 12 ♀♀.

The writer visited both herds, and the malformed calves were described independently by the two owners; in addition, the writer himself inspected one bull calf and four heifer calves showing the typical signs of achondroplasia. The following symptoms were characteristic: The head was comparatively short and broad with moderately bulging forehead, one to two cm long horn-knobs were usually present already at birth, more pronounced in the males than the females, in some cases causing difficult delivery, and the upper jaw was noticeably shorter than the lower jaw. The legs were comparatively short, particularly below knee and hock, the calves were standing on the tip of their toes, and on the hind-legs the toes were in most cases turned completely under (*cf.* Fig. 7). Gradually, however, the condition improved so the calves could walk fairly well after a week or two. The leg defect resembled very much the »flexed pastern» in newborn calves, described by MEAD, GREGORY and REGAN (1943), although in this case the length of the legs seems to have been normal. The achondroplastic calves were rather dull the first days after birth but became more lively and eager for food later on. All the calves, except six, were slaughtered before three weeks of age.

The calves were delivered after gestation of normal length, on an average 283 days. However, an excessive amount of amniotic fluid was noted in many cases. The bull calves showed consistently more pronounced symptoms of achondroplasia than the heifer calves. Apart from this difference, however, the manifestation of the head and leg malformations was rather uniform, and there were no difficulties in classifying the malformed and normal individuals.

Since the bull produced 25 calves with typical signs of achondroplasia in two different herds there could be no doubt that the malformation was hereditary. The numbers, 25 malformed and 28 normal calves, approximate very closely the 1 : 1 ratio, which would be expected if the bull carried a gene with pronounced effect in the heterozygous condition, and the cows were homozygous normals. It is extremely improbable that the gene is recessive and was carried by practically all the cows in the two herds. If the bull and all his mates had been heterozygous for such a gene the probability of obtaining 25 malformed and 28 normal calves would be only about 0,0005. However, the majority of the mates can not possibly be assumed to carry the same recessive gene, because no homozygotes had segregated out before, the cows belonged to different breeding lines, and the bull was not related to his mates. Even when 38 Hanorps Hero was mated to a Friesian cow he produced a malformed calf. However, since the bull and his parents were perfectly normal he could be expected to carry a dominant gene for achondroplasia only if a mutation had taken place in his own pre-germinal tissue at an early stage of the embryonic development.

It was decided to analyse the mode of inheritance by a breeding experiment, and for this purpose one bull and four heifer calves of the malformed type were bought to the Institute of Animal Breeding and raised there to maturity. At the time it was not possible to obtain a larger number of malformed animals. Unfortunately the bull proved to be sterile, and the heifers showed reduced fertility, and therefore the breeding experiment has not thrown any light on the problem.

THE EXPERIMENTAL ANIMALS.

The bull (Fig. 1) was kept until 2 years and 8 months of age when he suddenly died in the pasture field. He was growing very slowly, and the measurements presented in Table 1 show that he was a real dwarf for his age. The horns had normal length but were unusually heavy, and the forehead was rather broad, as shown by the prepared skull on Fig. 2. The distal parts of the legs were most abnormal; the pasterns were short and steep, and the hoofs were subnormal in size. Fig. 3 shows the skeletal parts of the left fore-leg of the bull in comparison to the right fore-leg of a normal bull of approximately the same age. The achondroplastic bull has considerably shorter but heavier bones than the normal animal. The phalangeal bones of the

hind leg were poorly developed; phalanx 3 was decidedly abnormal. An X-ray photograph of the left hind foot of the bull is presented in Fig. 4 together with a picture of a normal hind foot. The metacarpal bones of the achondroplastic animals are shorter but heavier than normal, especially in the metaphyseal region, and the phalangeal bones are also somewhat shorter.

TABLE 1. *Measurements of the achondroplasts in comparison with normal animals of the same breed, raised in the same herd; centimeters.*

	Achondroplasts		Normal animals	
Number of animals	4 ♀♀	1 ♂	14 ♀♀	6 ♂♂
Av. age when the measurements were taken, years	3 7/12	2 6/12	3 6/12	1 10/12
Height at withers, cm	126,5	122	130,8	132,8
» to olecranon, cm	70,5	63	74,8	74,2
» » tuber calcis, cm	47,3	47	49,5	49,7
Total length of animal from frontal eminence to tuber ischii	201,3	183	217,1	218,5
Length of body from first thoracic vertebra to tuber ischii	154,3	143	164,6	163,7
Depth of body (behind the withers)	68,3	67	72,4	72,8
Length of pelvis	51,0	53	54,6	55,5
Width at hips (tuber coxae)	48,3	44	54,2	50,3
» » thurls (trochanter major)	47,0	46	50,8	51,7
Chest circumference	183,3	182	195,6	197,3
Length of head	46,3	47	48,9	50,8
Width of head (at the supraorbital processes)	23,3	25	23,0	25,0
Head index (width in per cent of length)	50,3	53,2	47,1	49,2
Length of horns	26,8	22	26,0	24,0
Circumference of horns (at the base)	23,3	28	18,1	21,7

The bull was very timid, and when teased he would lie down as an opossum; even at 2 1/2 years he bellowed as a calf and not as a bull. After 1 1/2 years of age he was tried repeatedly with heifers in heat but never showed any noticeable desire to serve. When he was about 2 years it was tried to obtain semen by electric shocks, according to the method used successfully on rams (*cf.* GUNN, 1936), but no ejaculations could be produced. After the death of the bull the secretion in the epididymus was studied under the microscope and spermatozoa were found in small numbers. At this time it was not possible, however, to tell if the sperms had ever been motile and morphologically normal or not.

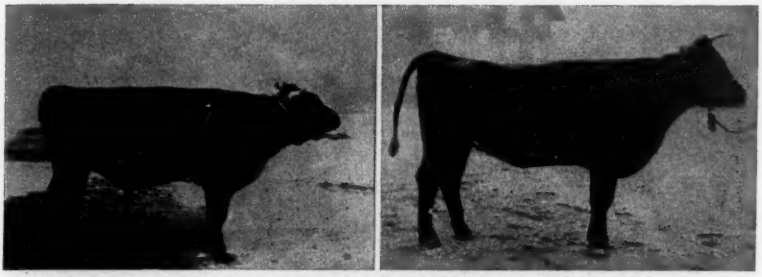

Fig. 1. Two achondroplasts: the bull at 2 years of age and the heifer No. 3 at 4 years.

The heifers were less abnormal in development and behaviour than the bull. All four had, however, short and broad heads (Fig. 5), heavy horns and comparatively short legs. Average measurements of these heifers are shown in Table 1 in comparison to the corresponding measurements of 14 heifers of the same age and raised in the same herd. The achondroplasts grew slower than the normals and did not reach the same size. At the time they were mated they were close to three years of age. They should have been mated about a year earlier but we were waiting for their half brother to develop sexual activity, which he never did. The achondroplastic animals were all stubborn and stupid in comparison to their herd mates. Since the bull was unable to serve, the heifers were run on the pasture together with a normal bull, in the summer of 1951, and in the autumn they were all diagnosed as pregnant. The following data on the animals may be of interest:

No. 1 was slaughtered in May 1952 because she was carrying a mummified fetus which she did not expel after repeated oxytocin injections. The fetus had apparently died after six or seven months of pregnancy, and it had probably been malformed although it was not possible to ascertain the degree or kind of malformation. The preserved skull and the right hind foot of the heifer are shown in Fig. 6.

No. 2 gave birth to a normal heifer calf the 5th of May 1952. In 180 days she has produced only 1757 kg milk with 4,02 per cent fat, and 6 months after calving she was almost dry. She has been served 6 times by fertile bulls but is not yet pregnant.

No. 3 resorbed her fetus and it has not been possible to get her pregnant afterwards. A photograph of this heifer, together with the bull, is shown in Fig. 1.

No. 4 delivered a stillborn but otherwise normal calf the 19th of

Fig. 2. The prepared skull of the achondroplastic bull (left) compared to a skull of a normal bull (N) of approximately the same age (2 years 8 months) and the same breed.

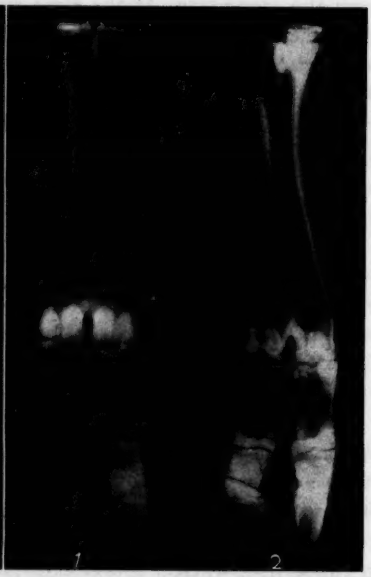

Fig. 3 (left). Skeletal parts of front feet: 1, normal bull; and 2, the achondroplastic bull. — Fig. 4 (right). An X-ray photograph of the left hind foot of a normal bull (1) and of the achondroplastic bull (2). The third phalangeal bones of the latter are decidedly subnormal in size.

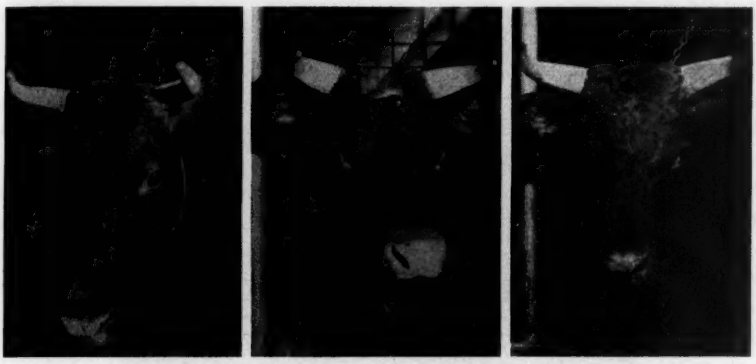

Fig. 5. Photographs of the heads of one normal cow in the station herd and of the achondroplastic cows 3 and 4, both about 4 years old.

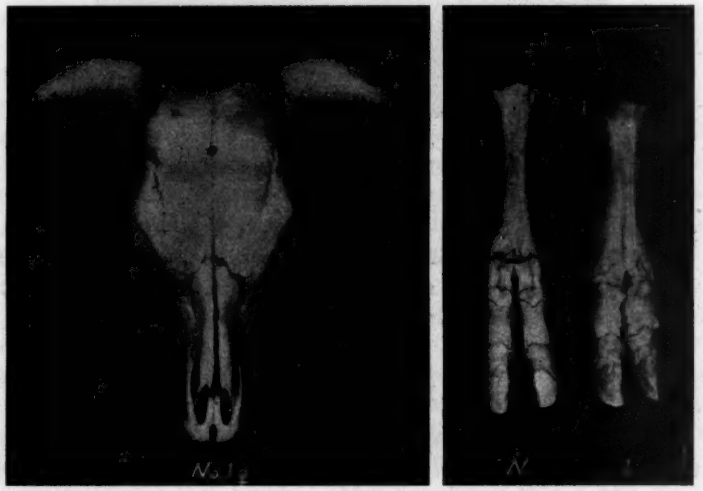

Fig. 6. Skull and right hind foot of the achondroplastic heifer No. 1, the latter compared to the corresponding skeletal parts of a normal heifer (N).

July 1952. Her milk yield was very low. She was slaughtered the 13th of October due to a foreign body which had penetrated through the diaphragm into the lungs. During her illness she re-developed the »flexed pastern» condition which was characteristic of the newborn calves (Fig. 7).

Fig. 7. Photograph of hind legs of the achondroplastic heifer No. 4, taken the day before slaughter, when she had re-developed her early calfhood character »flexed pasterns».

Only two of the experimental animals are still alive in the middle of October 1952, and it is rather uncertain whether or not it will be possible to get these animals pregnant.

DISCUSSION.

MØRCH (1941) states in his monograph on achondroplasia in man that the defect is always hereditary, that the causative gene is always dominant, and that achondroplasia frequently arises through mutation. In cattle five distinct types of achondroplasia were known before, and a sixth type is described in this paper. In three of the cases the responsible gene is completely recessive (one type is lethal, one sublethal and one relatively harmless); in another type the gene reduces the length of the head and legs in the heterozygotes but does not seem to decrease their viability (the Dexter cattle), whereas it is invariably lethal in the homozygotes; in a fifth type the only noticeable effect in the hetero-

zygotes is a marked reduction in the length of legs (»duck-legged» cattle), and the homozygotes are not known. The achondroplasts described in the present paper are probably heterozygotes. They are characterized by reduced length of head and feet, »flexed pasterns» on the hind legs during the early post-natal life, heavy horns when full-grown, and by a general reduction in the growth-rate, vitality and fertility. The effect of the responsible gene is manifold; it influences not only the development of the skeleton, changing the shape of the head and the proportions between trunk and legs, but it has also a profound influence on the physiology and psychology of the animals. The bull developed testicles of normal size but he was neither willing nor capable to serve the cows. He made slight attempts to masturbate but it is doubtful if he ever ejaculated. His type and behaviour suggested endocrine disturbances. Owing to his sudden death on pasture no study could be made of hypophysis or gonads. Although the heifers were less abnormal they also showed signs of endocrine disturbance, *e. g.* reproductive failures, small udders and low milk yield.

It is interesting to note that in our achondroplastic animals, as well as in the Dexter cattle, the proximal bones of the extremities are perfectly normal, the metacarpal and metatarsal bones are most reduced in length, and the phalangeal bones are also comparatively short; the hoof bones may be badly deformed. In the »duck-legged» cattle, however, all the long bones of the extremities seem to be reduced in length to about the same extent. In human achondroplasts it is mainly the proximal bones which are affected, as pointed out by MØRCH (1941) and BÖÖK (1950).

That new genes for different types of bovine achondroplasia, dominant as well as recessive, arise through mutation is generally acknowledged, and repeated mutations at the same locus are also known to occur, although data are lacking for an estimate of the mutation rate. BÖÖK (1952) has estimated the mutation rate in regard to human achondroplasia to 7×10^{-5}, based on 44.109 consecutive births at the University Hospital of Lund. — Our hypothesis that a new gene for bovine achondroplasia, with a pronounced effect in the heterozygotes, has arisen by mutation in the pre-germinal tissues of the bull 38 Hanorps Hero may seem far-fetched, but should not be too incredible. At any rate, it is the only explanation which the author finds plausible at the present time.

SUMMARY.

A new type of achondroplasia in cattle is described. One normal bull, mated to unrelated cows of various descent, produced 28 normal and 25 malformed calves. The latter had moderately bulging forehead, the upper jaw and the legs below knee and hock were reduced in length, and »flexed pastern» occurred usually on the hind feet. The malformations were more extreme in males than in females. The calves were born alive, and their viability during the first weeks after birth was approximately normal. One bull and four heifer calves were raised. They grew slower than normal animals; the bull was literally a dwarf, and never reached potency to copulate. The heifers were less abnormal in size, conformation and behaviour but their fertility as well as their milk yield after calving were subnormal. — It is assumed that the defective animals were heterozygous for a gene for achondroplasia, and that this gene had arisen by mutation in the pre-germinal tissues of the sire of the malformed calves.

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THE KINETOCHORE AND THE BIVALENT STRUCTURE IN THE LEPIDOPTERA

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I. THE KINETOCHORE IN THE LEPIDOPTERA.

WHILE the haploid chromosome number of most of the Lepidoptera studied cytologically is 29—31 (FEDERLEY, 1938, pp. 456—457; MAKINO, 1951), several cases are known where the chromosome numbers of closely related species of Lepidoptera differ even considerably from one another. Usually in such cases certain single species deviate greatly in chromosome number from the other closely related species. Such abrupt deviations of the chromosome number are known among the Lepidoptera in the following species pairs or groups, among others.

Rhopalocera.

Pieris brassicae, $n = 15$. The other investigated *Pieris* species (5 species), $n = 25$—26.

Leptidea sinapis, $n = 26$—41. *L. morsei*, $n = 54$. *L. duponcheli*, $n = 104$.

Argynnis ino, $n = 12$—14. The other investigated *Argynnis* (and *Brenthis*) species (12 species), $n = 28$—31.

Erebia tyndarus, $n = 8$. *E. ottomana*, $n = 40$. — These *Erebia* species are so closely related that they were not even distinguished as separate species until 1936 (LORKOVIĆ, 1948).

Erebia medusa and *E. medusa polaris*, $n = 11$. *E. oëme*, $n = 14$. The other investigated *Erebia* species (11 species), $n = 17$—29.

Genus *Lycaena* (in the old wider sense) (24 species), $n = 22$—26. *Lycaena bellargus*, $n = 45$. *L. coridon*, $n = 90$.

Bombyces.

Orgyia thyellina, $n = 11$. *O. antiqua*, $n = 14$. The other investigated *Orgyia* species (3 species), $n = 28$—30.

Dasychira selenitica, $n = 22$. *D. pudibunda*, $n = 87$.

Geometrae.

Biston hirtaria, $n = 14$. The other investigated *Biston* species (2 species), $n = 51$—56.

For the original literature on these chromosome numbers, see MAKINO (1951).

When studying the chromosome conditions of the Finnish species of the Geometrid genus *Cidaria*, I have encountered an entirely corresponding situation. So far, I have made a cytological study of 25 species of this genus (a more detailed account will be published later). 21 of these species have a haploid chromosome number of 27—32.

On the other hand, the haploid chromosome number of two closely related species of *Cidaria*, viz. *C. variata* SCHIFF. and *C. obeliscata* HB., is only 13 (Figs. 1—3 and 8). Out of the four other species of this subgenus (*Thera* STEPH.) occurring in Finland I have had two (*C. juniperata* L. and *C. firmata* HB.) available for cytological study so far. The chromosome number is 30 (Fig. 5) in the former and 19 (Fig. 6) in the latter. All these four species are systematically related and also fairly similar morphologically.

If the chromosome (bivalent) sizes in the four *Cidaria* species studied are compared with each other, it becomes apparent that they are much larger in the species with fewer chromosomes. The size difference of the chromosomes is really very considerable (*cf.*, for instance, Figs. 3 and 5). A corresponding size difference can be observed in all the cases mentioned above.

Various theories have been put forward as to how lepidopterous chromosome sets of this kind can be derived from each other. LORKOVIĆ (1941, 1948) expresses the opinion that such great deviations in the chromosome numbers represent cases of polyploidy. He bases this opinion on the fact that in certain genera of Lepidoptera (*Leptidea*, *Erebia*, *Lycaena*) the chromosome numbers are in fact multiples of the same basic number. WHITE (1946), on the other hand, does not consider polyploidy possible in these cases. Neither did FEDERLEY (1938, 1943, 1945) regard polyploidy as the reason for these deviations in chromosome numbers. He assumed that they are due to »fragmentation» or »fusion» of single chromosomes, with the result that single chromosomes in one species are represented by two or more elements in another.

This question is closely connected with the problem of the character of the kinetochore in lepidopteran chromosomes, a problem which has remained open until recent years. Thus, for instance, WHITE (1945, p. 177) remarks that we do not know anything about the kinetochore in the Lepidoptera. FEDERLEY (1943, 1945), however, basing his opinion on several arguments, came to the conclusion that in the Lepidoptera a non-localized kinetochore is to be found. He (1945, p. 9) particularly emphasized that »bei den Lepidopteren gibt es nichts, das darauf deuten würde, dass jedes Chromosom ein besonderes genau lokalisiertes

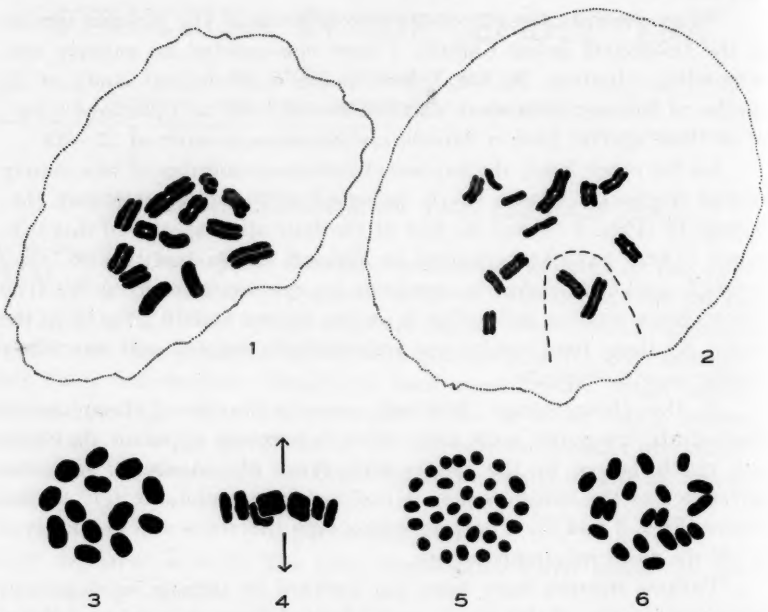


Fig. 1. *Cidaria obeliscata.* ♀, late diakinesis. — Figs. 2—4. *Cidaria variata,* ♀. Fig. 2. Late diakinesis. Fig. 3. First metaphase plate. Fig. 4. First metaphase plate in side view (part of the bivalents omitted). — Fig. 5. *Cidaria juniperata.* ♀, first metaphase plate. — Fig. 6. *Cidaria firmata.* ♀, first metaphase plate.

Zentromer besässe». (*Cf.* also SCHRADER, 1947, p. 142 and LIMA-DE-FARIA, 1949, pp. 433—434.)

In my opinion it is more than probable that the Lepidoptera in fact have a non-localized, or diffuse, kinetochore. Many cytological features found in the Lepidoptera can be understood only if their kinetochore is not localized. The great differences between the chromosome numbers of closely related species of Lepidoptera, pointed out in the preceding, are in my opinion, at least mostly, due to a »fragmentation» or »fusion» of the chromosomes, as FEDERLEY (*op. c.*) has suggested. (The details of the process by which this may take place remains outside the scope of this paper.) Such a process, again, is possible only if the chromosomes have a diffuse kinetochore. In this case the chromosome fragments would be viable and, on the other hand, the »fusion» of chromosomes — however it might take place — would not produce several localized kinetochores in the same chromosome. The idea of »fragment-

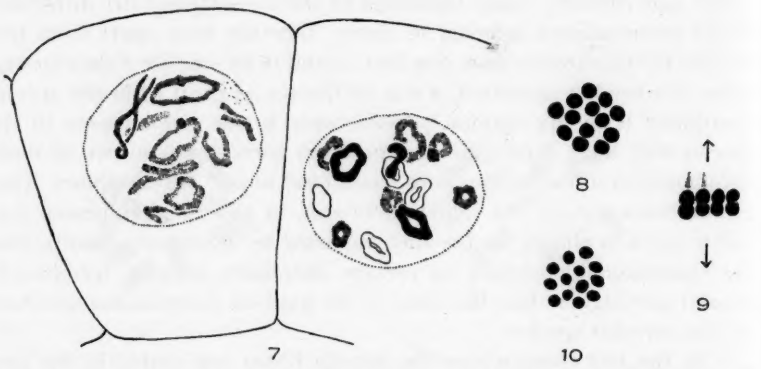
ation» and »fusion» is also supported by the considerable size difference in the chromosomes referred to above. Whereas even apart from this the size of the chromosomes has been found to be smaller if the chromosome number has increased, a size difference as great as in the species mentioned is in my opinion possible only if one chromosome in the species with large (and few) chromosomes corresponds to two or more chromosomes in the species with small (and many) chromosomes. That the chromosomes in the Lepidoptera can, in fact, be fragmented into viable parts is shown by the fact, reported by FEDERLEY (1943), that the chromosome numbers of certain *Dicranura* hybrids (*erminea* × *vinula*) are higher than the sum of the haploid chromosome numbers of the parental species.

In the few cases where the spindle fibres are visible in my preparations, it looks as if they really were attached to the chromosome more broadly than at one limited point (Fig. 9). LORKOVIĆ (1941, p. 173) mentions that in *Leptidea sinapis* as many as four spindle fibres may be attached to the largest single chromosomes.

II. THE CHIASMATA IN THE LEPIDOPTERA.

The question of whether or not chiasmata are formed in the bivalents of the Lepidoptera has also remained an open one until fairly recent years. MAEDA (1938) has observed distinct chiasmata in the males of *Bombyx mori*, but is of the opinion that chiasmata are not formed in the female. However, WHITE (1945, p. 193) shows, on the basis of MAEDA's figures, that in the female silk moth a single chiasma occurs in each bivalent, near the end. This chiasma may be completely terminalized, so that the two chromosomes which form the bivalent are in end-to-end contact. Although FEDERLEY (1943, p. 242) mentioned having now and then found distinct cross and ring bivalents in species with large chromosomes, like *Dicranura vinula*, he was still in 1943 of the opinion that »Chiasmata kommen überhaupt nicht in der Oogenese der Lepidopteren vor, und in den kugeligen Chromosomen der Spermatogenese ist dies noch weniger der Fall» (p. 245). However, a little later (1945) he was able to find distinct chiasmata in the spermatogenesis of *Trichiura crataegi*, a moth species with large chromosomes, and to analyse them.

That entirely contrary opinions concerning the formation of chiasmata in the bivalents of the Lepidoptera have been expressed so recently depends at least in part on the small size of the chromosomes and their



Figs. 7—10. *Cidaria variata*, ♂. — Fig. 7. Early and late diakinesis. — Fig. 8. First metaphase plate. — Fig. 9. First metaphase plate in side view. — Fig. 10. Second metaphase plate. — In the left cell in Fig. 7 and in Fig. 9 part of the bivalents omitted.

spherical form which make it difficult to observe the chiasmata. As the species with larger chromosomes can offer better opportunities, let us study the chiasma conditions in the bivalents of *Cidaria variata* and *C. obeliscata*.

The only preparations of spermatogenesis that I have obtained, are of *Cidaria variata*. Fig. 7 shows the early and the late diakinesis. We see that most of the bivalents are typical ring bivalents which seem to have two terminalized chiasmata. The nuclei have very few rod bivalents (for instance, in the right-hand cell of Fig. 7 only one), which seem to have one chiasma. In my opinion these bivalents so closely resemble bivalents having chiasmata that chiasmata are undoubtedly formed in the male Lepidoptera. It is not possible to ascertain from my preparations whether the chiasmata are formed at the ends of the bivalents or at some other place becoming rapidly terminalized. For instance, in the bivalent on the extreme left of the left-hand cell of Fig. 7 there seems to be, in addition to a terminal chiasma, an interstitial chiasma, which points to the latter alternative. At metaphase the observation of the chiasma conditions is more difficult because of the strong contraction of the chromosomes.

As regards oogenesis, I have been able to investigate the chiasmata both in *Cidaria variata* and in *C. obeliscata*, although only in diakinesis and at first metaphase. In regard to their chiasmata (and also in other cytological respects) these spp. are entirely similar. In dia-

kinesis each bivalent has one quite terminal chiasma, the site of which, it is true, is no longer clearly visible in all bivalents, although it can be clearly seen in some (Figs. 1—2). Neither is it any longer possible to conclude from preparations of such late phases in what place the chiasma has originally been formed. If in the female Lepidoptera it is originally localized at the end of the bivalent, this would explain why crossing-over has not been experimentally established in certain female Lepidoptera (for further details see, *e. g.*, WHITE, 1945, p. 193).

What has been said above seems to indicate that in the Lepidoptera the chiasma frequency per bivalent is considerably higher in the male than in the female.

The observations presented above are in full agreement with what is seen from the figures of some earlier studies on the cytology of the Lepidoptera. Thus in them also the majority of the bivalents in the diplotene and diakinesis of spermatogenesis are ring bivalents (*e. g.*, DEDERER, 1907, Figs. 23—27; BUDER, 1915, Fig. 36; CRETSCHMAR, 1928, Taf. IV, Abb. 29). In the diakinesis of oogenesis, on the other hand, only bivalents with only one terminal chiasma can be seen (*e. g.*, FEDERLEY, 1931, Abb. 8—9).

III. THE ORIENTATION OF THE BIVALENTS AT FIRST METAPHASE IN THE LEPIDOPTERA.

If we study the form of the bivalents at first metaphase of the spermatogenesis of the Lepidoptera, we find that they usually have a rounded outline in polar view (*cf.* Fig. 8). In side view the bivalents clearly have a dumb-bell shape, the long axes being parallel with the direction of the spindle (Fig. 9). This can also be observed from many other studies on spermatogenesis of the Lepidoptera. This orientation of the bivalents is very clearly seen in such moth species as, for instance, *Trichiura crataegi*, in which the more detailed structure of the bivalents is still visible at metaphase (FEDERLEY, 1945, Fig. 7—8). This shows that in the male Lepidoptera the chiasmata break in the first meiotic division, which is thus the reduction division. The meiosis of the male Lepidoptera is thus prereductional.

Some authors (*e. g.*, FEDERLEY, *op. c.*) mention that at the first metaphase of oogenesis of the Lepidoptera the form of the bivalents in polar view is more elongated than in spermatogenesis. This point has not, however, attracted any particular attention. If we study the orientation of the bivalents in the female Lepidoptera, the explanation of the

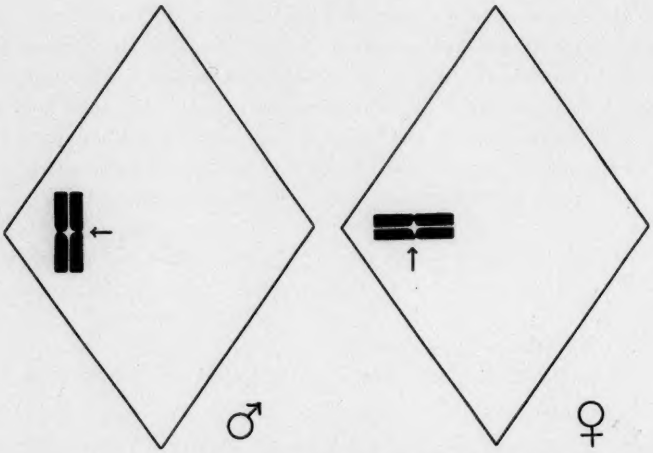


Fig. 11. Scheme of the orientation of the bivalents in the spindle at first metaphase in the Lepidoptera (in side view). Left in the male, right in the female. The arrow shows the site of the chiasma.

difference in shape is seen. In oogenesis the bivalents are orientated so that their long axes lie at an angle of 90° to the direction of the spindle (Fig. 4). This means that in polar view the bivalents have an elongated or even a dumb-bell shape (Figs. 3 and 6). This form of the bivalents of the female is very clearly seen in many figures published earlier (*e. g.*, SEILER, 1914, Textfig. 3 and 10; FEDERLEY, 1931, Abb. 4; 1943, Fig. 2). The fissure between the two halves of a bivalent cannot represent any other formation but a terminal chiasma. This means that the female Lepidoptera have an auto-orientation and that in such bivalents the chiasmata are preserved until the second division, which again means that the meiosis of the female Lepidoptera is postreductional.

If we compare the diakinetic bivalents of female Lepidoptera (Figs. 1—2) with the corresponding bivalents of, for instance, aphids (RIS, 1942, Fig. 81; *cf.* also RIS, Figs. 11—12) which are known to have diffuse kinetochores and to divide postreductionally, we find a complete similarity. The same similarity can be observed if the corresponding metaphase bivalents are compared in side view (Fig. 4; FEDERLEY, 1938, Fig. 27; RIS, *op. c.*, Figs. 17 and 19).

The exceptional orientation of the bivalents of female Lepidoptera in the metaphase has caused many investigators to assume that they

have no chiasmata and that the secondary split between the chromatids of one chromosome is the primary split between conjugated homologous chromosomes. The terminal location of the chiasma has led to the latter assumption also where diakinetic tetrads have been concerned. From the manner of orientation of the bivalents it is evident that the elimination chromatin typical of the oogenesis in the Lepidoptera is formed in the secondary split between the chromatids of one chromosome.

The orientation of the bivalents in the Lepidoptera resembles that of certain bugs (Hemiptera Heteroptera) in which HELENIUS (1952) has similarly found prereduction in the male and postreduction in the female.

Some figures of KLINGSTEDT's (1931, *e. g.*, Figs. 8, 10 and 11 a, g) seem to indicate that the bivalents in caddis flies (Trichoptera) orientate in the same way as in the Lepidoptera.

Material and methods. — The material used was collected in South Finland: *Cidaria variata*, *C. obeliscata* and *C. firmata* in Helsinki and Porvoo, *C. juniperata* in Heinola. The fixed testes, available only from *C. variata*, were taken from adult male caterpillars. Of the females ovaries with mature eggs were fixed.

The material was fixed in CARNOY's fluid (6 : 3 : 1) and sectioned at a thickness of 15μ. The preparations were stained in HEIDENHAIN's iron-hematoxylin. The drawings were made at the level of the microscope stage with an Abbé camera lucida, using a $\times$ 120 immersion objective and a $\times$ 25 ocular. Since the drawings were reduced in size to two-thirds of the original in making the printing-plates, the magnification is now about 2000 $\times$.

SUMMARY.

(1) The kinetochore in the Lepidoptera is a diffuse kinetochore, as suggested earlier by FEDERLEY.

(2) In the Lepidoptera chiasmata are formed in the bivalents of both the male and the female. In general the females have only one terminal chiasma in their bivalents in diakinesis and at first metaphase.

(3) Meiosis in the Lepidoptera is prereductional in the male but postreductional in the female.

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INVESTIGATIONS ON SOME TUBER-BEARING SOLANUM HYBRIDS

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INTRODUCTION.

IN 1942 I made a cross between the two species *Solanum acaule* var. *subexinterruptum* BITT. and *S. tuberosum* L. It is very difficult to cross these tetraploid ($2n = 48$) species directly, but after chromosome doubling of *S. acaule*, hybrids are easily obtained, provided that the latter is used as the pistillate parent and that pollen is taken from a suitable variety of *S. tuberosum*. Some investigations of these hybrids have been reported in an earlier paper (LAMM, 1945, pp. 90—92). Further studies reported here have shown that the reciprocal cross always fails, but that the hexaploid F_1 hybrids can be reciprocally crossed with *S. tuberosum* and probably also with *S. acaule*. The two pentaploid backcross progenies to *S. tuberosum* hereby obtained are quite different with respect to the male fertility, which is »normal» in $F_1 \times S.$ *tuberosum*, while the reciprocal combination gives absolutely male sterile plants.

Three remarkable features, which will be discussed in this paper, are thus characteristic of this interspecific cross, *i. e.* the one-way incompatibility, the failure of the $4x \times 4x$ as opposed to the success of the $8x \times 4x$ matings, and the difference in male fertility between the reciprocal backcrosses of the F_1 hybrids to *S. tuberosum*.

THE PARENTAL SPECIES.

S. acaule (see Fig. 1) and *S. tuberosum* belong to different groups of the tuber-bearing *Solanum* species. Of the numerous characters distinguishing these two species, those of importance for the present investigation concern mainly the breeding mechanism and kind of polyploidy. Probably both species can be regarded as segmental allopolyploids (*cf.* STEBBINS, 1950, p. 320), but relatively to each other *S. tuberosum* is auto-, *S. acaule* allopolyploid.

Both cytological and genetical evidence agree in assigning a comparatively pronounced degree of autopolyploidy to *S. tuberosum*

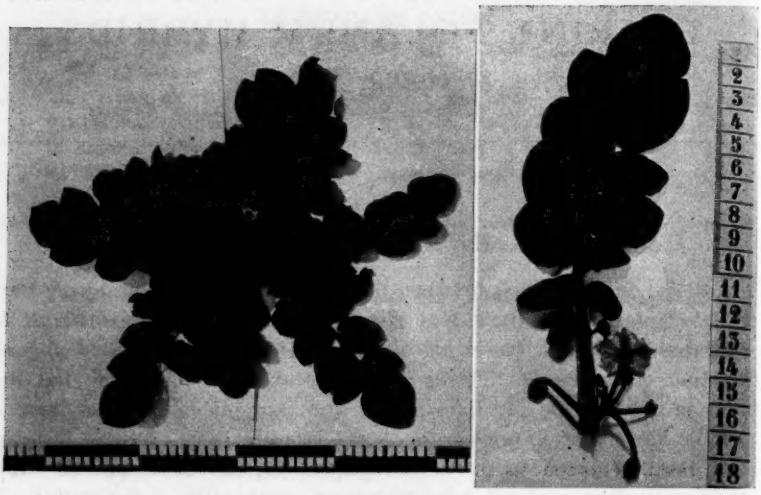

Fig. 1. Colchicine induced octoploid plant of *Solanum acaule* var. *subexinterruptum* BITT.

(*cf.* LAMM, 1945, p. 58 and pp. 96—100), but on the other hand some authors (*e. g.* THOMAS, 1946) have pointed out the allotetraploid nature of this species. The allopolyploid nature of *S. acaule* is inferred mainly from the regular bivalent pairing, which, because of the meiotical behaviour of the induced *S. acaule* octoploids, seems not to be merely a genotypically controlled two-by-two pairing in an intrinsic autopolyploid (*cf.* LAMM, 1945).

So far no radical structural changes capable of seriously affecting pairing in species hybrids, have been observed in the tuber-bearing *Solanum* species (*cf.* SWAMINATHAN, 1951). Since pachytene studies have not been made, this assumption is, however, only weakly founded. In some interspecific crosses within the closely related genus *Lycopersicon* BARTON (1951) found that in spite of a comparatively good pairing the pachytene morphology of the species concerned deviate markedly. In the evolution of the tuberous *Solanum* species gene mutations are probably most important but there may also exist cryptic structural differentiation.

S. acaule is perfectly self-compatible. I have found that well developed berries sometimes contain more than 400 seeds, although the mean number of seeds in five such berries was 326. Octoploid plants

of *S. acaule* (Fig. 1) produced by the colchicine method were comparatively vigorous and fertile. Here the most fertile berries contained 106 seeds, the average of eighteen fruits being 74. The mean percentage of good pollen had decreased from 96 in the tetraploid to 73 in the octoploid.

S. tuberosum contrary to *S. acaule* is mainly allogamous. Most of the related diploid species show homomorphic incompatibility controlled by *S* alleles, although in some cases the crossing tests did not quite follow the simple behaviour outlined in the oppositional factor hypothesis (*cf.* CARSON and HOWARD, 1942, and PUSHKAR NATH, 1945). *S. tuberosum* is to a certain degree self-compatible, which might be expected, since in autotetraploids incompatibility frequently breaks down (*cf.* LEWIS, 1949), but in some respects it still reveals its allogamous nature (*cf.* LAMM, 1945, pp. 103—104 and p. 111).

Most varieties of *S. tuberosum* are partially male sterile. Complete female sterility is rare, but there are many entirely male sterile *S. tuberosum* varieties. For my crosses I have used the comparatively fertile varieties Deodara, Parnassia and Pepo. Successful crosses with octoploid *S. acaule* were obtained with Deodara as the male partner. In the backcrosses Parnassia and Pepo were also used, partly in order to avoid inbreeding, partly because of their resistance to wart disease. These three varieties have all been raised from the cross Deutsches Reich × Jubel (see v. RATHLEF, 1932), and, from the inbreeding point of view, the choice is thus less suitable. Investigations by DREMLIUG (1937), STELZNER (1943) and SWAMINATHAN (1951) have shown that *S. acaule* can be successfully crossed with several *S. tuberosum* varieties.

For Deodara, Parnassia and Pepo the mean percentage of apparently good pollen during 1946—1949 was 50, 49 and 37. In Deodara three good berries contained 163, 185 and 192 seeds (average 180).

THE F_1 HYBRIDS.

With octoploid *S. acaule* as mother crosses with *S. tuberosum* variety Deodara are comparatively successful, and it is not necessary to graft the female partner on tomato, as I did in 1942. In five berries from this mating the seed content varied between 12 and 39 (average 25) as is shown in Table 4 (Cross No. 1). I never succeeded in getting seeds of this interspecific cross when using Parnassia as the male partner. High fertility of a *S. tuberosum* variety therefore does not mean that it is also suitable for this mating.

The pollen-tube growth was studied in slides of styles and ovaries from reciprocal crosses between *S. tuberosum* and *S. acaule*. When using the varieties Deodara, Parnassia and Pepo as pistillate parents I observed that the pollen-tubes from 4*x* and 8*x* *S. acaule* plants were inhibited in the *S. tuberosum* styles and that the eggs were never fertilized. The reciprocal combination was only investigated with Parnassia. Already 48 hours after the pollination some of the *S. acaule* embryosacs had been fertilized, and after 144 hours the hybrid endosperm had developed considerably, and small embryos were discerned. It is interesting to note that embryos were obtained both with 4*x* and 8*x* *S. acaule* plants as pistillate parents, and that they were formed also by using pollen of the variety Parnassia, which was otherwise incompatible as regards the seed production.

In selfed flowers of tetraploid *S. acaule* the early development of endosperm and embryo is faster than in the hybrid embryosacs. It is also faster than in octoploid *S. acaule*.

TABLE 1. *Numbers of seeds in some good berries harvested from three hexaploid (6x) F_1 hybrids from the cross S. acaule (8x) × S. tuberosum (4x), var. Deodara.*

Cross No.	Source of pollen	Seeds in one fruit of the F_1 hybrids			Average No. of seeds
		43/42	43/45	43/49	
2	F_1 (6*x*) open pollin.	133	147	193	158
3	» » selfed	98	55	78	77
4	*S. demissum* (6*x*)	134	133	126	131
5	*S. tuberosum*, Deodara (4*x*)	85	96	57	79
7	*S. acaule* (8*x*)	0	0	0	0
8	» » (4*x*)	5	0	0	2
9	*S. Rybinii* (2*x*)	3	1	1	2

The amount of viable seed obtained in *Solanum* crosses is obviously dependent on a number of factors such as the gametic fertility, the growth of the pollen-tubes, the proportion of ovules fertilized, and the proportion of ovules collapsed (the collapse probably being due mainly to the so-called somatoplastic sterility).

The F_1 hybrids have been described by LAMM (1945), who also studied their cytological behaviour. The somatic chromosome number (see Table 2) varied from 70 to 74 (average 72). The percentage of stainable pollen varied between 59 and 83. Three of the numerically balanced ($2n = 72$) hybrids, *i. e.* 43/42, 43/45 and 43/49, were tested in

TABLE 2. *Somatic (2n) numbers of chromosomes in F_1 and F_2 of the cross S. acaule (8x) × S. tuberosum (4x) and of the first and second backcrosses to S. tuberosum (4x). The basic chromosome number x=12.*

Hybrid progenies			Backcross progenies (see Fig. 2)					
	F_1	F_2		BC1a	BC1b		BC2a	BC2b
2n	Freq.	Freq.	2n	Freq.	Freq.	2n	Freq.	Freq.
						48	5	—
						49	4	1
68	—	2				50	8	—
69	—	—				51	8	1
70	3	8	58	2	—	52	11	—
71	—	—	59	5	1	53	18	4
72	10	6	60	21	13	54	22	6
73	4	4	61	4	3	55	11	—
74	1	1	62	3	1	56	6	1
75	—	1	63	—	1	57	—	2
						58	1	1
						59	1	—
Total	18	22		35	19		95	16
Mean (2n)	72,0	71,3		60,0	60,3		52,8	54,0

various intra- and interspecific crosses. The mean percentage of stainable pollen in these hybrids was 71, and the figure varied only slightly from one plant to another.

The content of viable seed in berries from these three hybrid plants varied considerably with the source of the pollen (Table 1). Fruits of the crosses Nos. 2—5 were readily obtained, while rather fairly many of the flowers had to be pollinated in order to get the berries of the crosses Nos. 7—9. Cross No. 7 appeared to be quite incompatible.

Of the crosses Nos. 3—5, only 10 pollinations of each were made with practically complete success. The three berries of cross No. 9 were obtained from 10 pollinations, while of the crosses Nos. 7—8, about 40 pollinations of each gave only one fruit in cross No. 8.

As male parents in crosses with the variety Deodara the F_1 hybrids gave only four fruits containing 6—32 seeds (Table 4, Cross No. 6), although numerous pollinations were made. The reciprocal cross gave a much larger seed production (Table 4, Cross No. 5).

The somatic chromosome numbers have been counted in some plants chosen at random out of the progenies raised from the crosses listed in Table 1. Some results of these determinations are given in Table 2. Most of the effective F_1 gametes seem to have a number of chromosomes equal to, or only slightly deviating from, 36. The first

TABLE 3. *Pollen fertility in the progenies of Fig. 2.*

Progenies (see Fig. 2)	Total numbers of plants	Numbers of non-flowering plants	Numbers of flowering plants: abs. male ster.	partially male ster. per cent good pollen: >0 — 30	— 60	— 90	Mean fert. of flowering plants
F_1	33			7	26		69
BC1a	69	3	2	13	35	16	45
BC2a	40	18		4	16	2	41
BC3a	37	2		14	16	5	37
BC4a	86	15	2	8	29	32	54
BC4c	120	27	38	28	19	8	19
Test a	155			14	114	27	49
Test c	50	19	31				0
BC1b	19		19				0
BC2b	10	2	6	2			4
BC3b	20	5	9	4	2		7
BC4b	10	3			5	2	50
Test b	10	3	7				0

generation backcross families (BC1a—1b) showed that 63 per cent of the gametes had this number, while, according to LAMM (1945, p. 91), only about 30 per cent of the second metaphase plates in the PMC's of the F_1 plants are numerically balanced. The somatic chromosome numbers in the progenies raised from the crosses Nos. 4, 8 and 9 of Table 1 support this conclusion (results unpublished).

TABLE 4. *Seed-content of berries from the crosses of Fig. 2.*

Cross No.	Parents ♀ × ♂	No. of berries	Seed content min.—max.	average
1	A′ × T	5	12— 39	25,4
5	F_1 × T	8	57—121	84,7
10	BC1a × T	22	8— 53	29,9
12	BC2a × T	38	4—154	38,0
14	BC3a × T	24	8— 76	23,3
15	T × BC3a	32	8—210	81,9
17	BC4a × F_1	32	5— 77	38,5
18	BC4c × F_1	11	12—111	33,6
6	T × F_1	4	6— 32	17,3
11	BC1b × T	6	1— 22	7,2
13	BC2b × T	2	35— 75	55,0
16	BC3b × T	6	25— 88	44,0
19	BC4b × F_1	1	—	12,0

THE BACKCROSSES TO S. TUBEROSUM.

Repeated backcrosses to *S. tuberosum* were made in accordance with the scheme in Fig. 2. In the first backcross generation mainly pentaploid plants were obtained (see Table 2). When F_1 was used as the pistillate parent the plants of the pentaploid progeny were rather fertile (Table 3, BC1a; Table 4, Cross No. 10), but the plants of the reciprocal crossing were completely male sterile (Table 3, BC1b) and in addition were poor in seed production (Table 4, Cross No. 11). A similar case has been described by LAMM (1941, 1945) in reciprocal crosses between the pentaploid species *S. curtilobum* and the tetraploid *S. tuberosum*.

In the male sterile BC1b plants meiosis is irregular. The second division is inhibited. During the first division there is often no pairing, and the univalents are either irregularly distributed or arranged in pseudo-homotypic plates. The tapetum of the anthers is often abnormal. This type of meiosis has been described in the *S. tuberosum* $\times$ *S. curtilobum* hybrids (LAMM, 1941, 1945).

The distribution of chromosome numbers within the progenies BC2a and BC2b is given in Table 2. Hybrids with intermediate chromosome numbers are the most common. The distribution is rather similar to that given by LAMM (1945) for the progeny of a $5x \times 4x$ cross between *S. curtilobum* and *S. andigenum*. As in the last mentioned progeny, in the two progenies of the present investigation there was no correlation between chromosome number and pollen fertility. Unfortunately no attempts were made to cross *S. tuberosum* with BC1a. It is very likely that also this mating would have given a male sterile progeny.

By repeated backcrosses I wanted to introduce the genome of *S. acaule* in the cytoplasm of *S. tuberosum*. Although 460 flowers of the BC1b plants were pollinated with pollen from *S. acaule* ($4x$) and 100 with pollen from *S. acaule* ($8x$), no berries were obtained. The BC1a plants were also incompatible with pollen of *S. acaule* ($4x$). Here 140 pollinations were tried without success. As already mentioned, the crossing between F_1 and *S. acaule* is possible though difficult (see Table 1, Cross No. 8). Pollen-tubes of *S. acaule* are probably inhibited in the styles of the BC1a—b plants, just as they are in the pure *S. tuberosum* styles.

A gradual increase of the fertility was obtained by repeated backcrosses of the BC1b plants to *S. tuberosum* and selection of the most

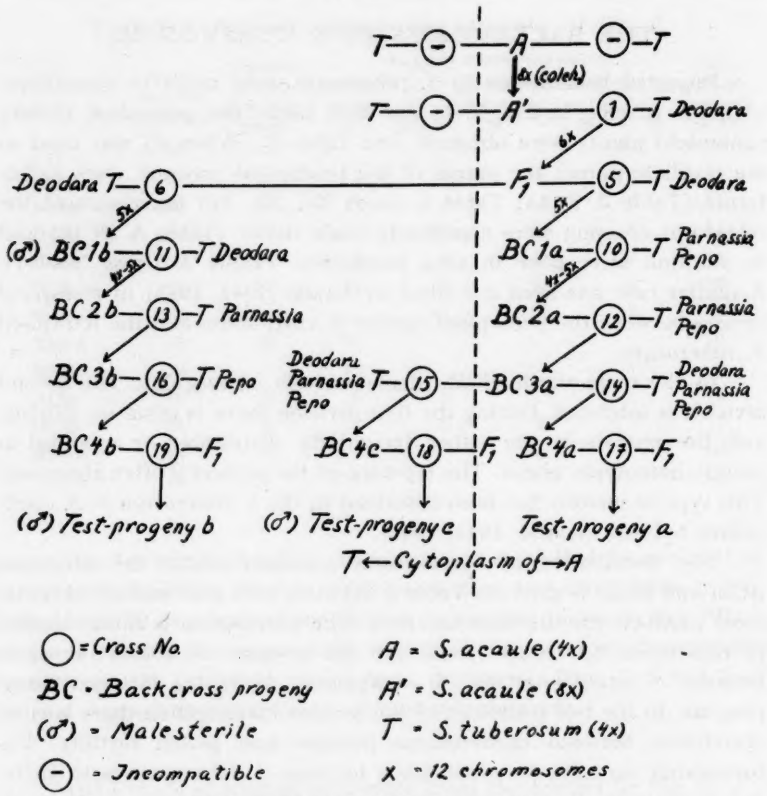
T — ⊖ — A — ⊖ — T
4x (colch.)
T — ⊖ — A' — 1 — T Deodara
6x
Deodara T — 6 — F1 — 5 — T Deodara
5x
5x
(♂) BC1b — 11 — T Deodara
BC1a — 10 — T Parnassia Pepo
4x-5x
4x-5x
BC2b — 13 — T Parnassia
BC2a — 12 — T Parnassia Pepo
BC3b — 16 — T Pepo
Deodara Parnassia T — 15 — BC3a — 14 — T Deodara Parnassia Pepo
Deodara Parnassia Pepo
BC4b — 19 — F1
BC4c — 18 — F1
BC4a — 17 — F1
(♂) Test-progeny b
(♂) Test-progeny c
Test-progeny a
T ← Cytoplasm of → A
⊖ = Cross No.
A = S. acaule (4x)
BC = Backcross progeny
A' = S. acaule (8x)
(♂) = Malesterile
T = S. tuberosum (4x)
⊖ = Incompatible
X = 12 chromosomes

Fig. 2. Scheme of crosses (see text).

fertile plants (Table 3, BC2b—4b; Table 4, Crosses Nos. 11, 13 and 16). Some of the BC4b plants were as male fertile as the most male fertile varieties of *S. tuberosum*.

The pollen fertility of 183 varieties of *S. tuberosum* was evaluated by v. RATHLEF (1932). Of these, 117 had less than 30 per cent good pollen. They were practically useless as male parents. According to v. RATHLEF (1932), the pollen fertility in *S. tuberosum* rarely exceeds 60 per cent. I therefore used the 30 and 60 per cent fertility as grouping intervals in Table 3. However, the mean fertility of the flowering plants was calculated from separate determinations made in the individual plants. In some progenies the frequency of non-flowering plants is high and is often caused by an early abscission of the flower buds. A few of

the non-flowering plants might possibly have produced fertile flowers if they had grown under more favourable conditions. The mean fertility of Table 3 is thus somewhat ill-founded.

By a series of crosses (see Fig. 2) *S. tuberosum* chromosomes were introduced into the cytoplasm of *S. acaule*. In the successive backcross generations the plants were selected for seed and tuber production, so that the backcross progenies do not represent a real random distribution of genotypes. The reason for this selection was partly lack of space in the fields, and partly the main purpose of these backcross studies, *i. e.* to find out if a relatively high fertility could be obtained in plants by combining the cytoplasm of *S. acaule* with the chromosomes of *S. tuberosum*. This seems to be possible, since in BC4a several plants had more than 60 per cent stainable pollen (see Table 3) and a farily good seed production (see Table 4). Although the agricultural value of the hybrids has not been tested, some plants in the late backcross progenies appeared to be of some interest from this point of view.

One possibility that must not be overlooked is that perhaps certain *S. tuberosum* genes never occur in homozygous condition in the cytoplasm of *S. acaule*. Possibly these genotypes are removed, *e. g.* through the mechanism of somatoplastic sterility. If this assumption is true, the most fertile plants of the backcross progenies would perhaps contain relatively more of the *S. acaule* genes than the less fertile ones. These plants, when backcrossed as pistillate parents to *S. tuberosum*, would give comparatively fertile progenies, while in the reciprocal matings they would give progenies somewhat more sterile than those obtained from their less fertile siblings.

In order to test this hypothesis cross No. 15 between *S. tuberosum* and BC3a was performed. A comparison of the pollen fertility of the reciprocal families BC4a and BC4c did not reveal the correlation expected. However, since only eight reciprocal combinations were studied, no definite conclusions can be drawn. BC4c was much more male sterile than BC4a (see Table 3), which indicates that the cytoplasm of *S. tuberosum* contributes to the development of male sterility.

Finally a series of »test crosses» was made (see Fig. 2). As expected, both cross No. 19 (BC4b $\times$ F_1) and cross No. 18 (BC4c $\times$ F_1) gave completely male sterile plants in their progenies (see Table 3). The male fertility of the progenies raised from cross No. 17 (BC4a $\times$ F_1) was comparatively high and completely male sterile plants were absent.

By another series of crosses the genes of *S. tuberosum* were introduced into the cytoplasm of *S. Rybinii*. Of this diploid species artificial

tetraploids were produced by the colchicine method (LAMM, 1945). One of these tetraploid plants was crossed with Deodara. By repeated backcrosses a BC4 generation was obtained. Plants within this progeny and with a high pollen fertility were crossed as pistillate parents with hexaploid F_1 hybrids between *S. acaule* and *S. tuberosum*. The progeny was fairly fertile. There is thus no interaction giving rise to male sterility between the cytoplasm of *S. Rybinii* and certain genes from *S. acaule* or *S. tuberosum*.

DISCUSSION.

STERILITY.

The literature on the sterility of *S. tuberosum* and some of its relatives is rather extensive (see KOOPMANS, 1951). Although several kinds of haplontic sterility are known, diplontic sterility seems to be the most important one. In some cases a correlation was observed between aberrant tapetal development and aberrant meiotic behaviour (see LAMM, 1945). One kind of diplontic sterility given special attention in this paper is the male sterility caused by the interaction between the cytoplasm of *S. tuberosum* and certain genes of *S. acaule*. Similar cases have been found in crosses between the former species and some other species, *i. e. S. curtilobum* (LAMM, 1941, 1945), *S. antipoviczii* (IVANOV. 1939) and *S. demissum* (JOHNSTONE, 1941). In the male sterile *Solanum* hybrids the first meiotic division is aberrant in various respects and the second division is entirely inhibited. Tetrads do thus not form. In male sterile plants of other genera the degeneration often occurs after the formation of the tetrads.

Numerous cases of male sterility produced by combined cytoplasmic and genic inheritance are recorded. The sterility genes and cytoplasm may belong either to the same species as in *Allium* (JONES and CLARKE, 1943) and *Beta* (OWEN, 1945) or to different species as in *Nicotiana* (EAST, 1932) and *Epilobium* (MICHAELIS, 1933). Apart from the appearance of the sterility cytoplasm and genes in different species, the *Solanum* cases seem to be more closely comparable to those in *Allium* and *Beta*. In *Solanum*, however, a thorough genetical analysis was not made, and the comparisons are therefore vague.

Cytoplasmic differences may perhaps also exist within the species *S. tuberosum*. If this is true, it would help to explain the tendency of a maternal inheritance of the pollen sterility observed in some variety crosses (*cf.* FINEMAN, 1947).

In *Nicotiana* (EAST, 1932) the sterility cytoplasm belongs to the self-compatible species *N. Langsdorffii*. This cytoplasm gives male sterility with any two self-incompatibility factors from *N. Sanderæ*. This case is thus very different from *Solanum*. In *Epilobium*, F_1 of *E. hirsutum* $\times$ *E. luteum* is completely male sterile, but sterility is also gradually obtained by the introduction of an increasing amount of *E. hirsutum* genes into the cytoplasm of *E. luteum*. The differences between the *Solanum* and *Epilobium* cases are apparent from a comparison of the fertility of corresponding reciprocal progenies (see Table 3, BC4a and BC4c and MICHAELIS, 1933, p. 70).

In this connection it should be mentioned that the BC4a, 4b and 4c plants of *Solanum* may contain several *S. acaule* chromosomes. In comparable backcrosses COOPER and HOWARD (1952) have shown that the formation of seedlings with the exact tetraploid chromosome number is a fairly slow process. Yet we do not know if the introduction of all the genes of *S. tuberosum* into the cytoplasm of *S. acaule* would give rise to male fertile plants.

As mentioned in the introduction, the cross *S. acaule* $\times$ *S. tuberosum* was only successful when octoploids of the former species were used. A parallel to this behaviour was studied by COOPER and BRINK (1945) in the cross between the two diploid species *Lycopersicon pimpinellifolium* and *L. peruvianum*. Here, unlike the $4x \times 2x$ crosses the $2x \times 2x$ crosses fail. In the later crosses they found a disturbed seed development due to so-called somatoplastic sterility. I have often noticed the occurrence of plump seeds in the ripe *Solanum* berries. Unfortunately, it is practically impossible to determine the amount of zygotic elimination due to plump seeds, which is a considerable disadvantage in studies of sterility and incompatibility in *Solanum*.

INCOMPATIBILITY.

Intra- and interspecific incompatibility, often of the one-way type, is fairly common among the tuberous *Solanum* species. Most species crosses have been made in order to investigate the crossability with *S. tuberosum*, either by direct crossing or by using a hybrid as a genetical bridge (*cf.* BLACK, 1944). In these investigations, however, the genetical mechanism separating different species was practically neglected.

The section *Tuberarium* contains both self-compatible and self-incompatible species. The former category includes, among others, the species of the subsection *Acaulia* (*e. g. S. acaule*), *Longipedicellatæ* (*e. g.*

the tetraploid species *S. longipedicellatum*, *S. antipoviczii* and *S. ajuscoense*), *Demissa* (*e. g.* the hexaploid *S. demissum*), and *Polyadenia* (*e. g.* the diploid *S. polyadenium*). Most species within the subsection *Commersoniana* (*e. g.* the diploid *S. chacoense*) and *Tuberosa* (*e. g.* the diploid species *S. phureja* and *S. Rybinii* and the tetraploid species *S. tuberosum*) are self-incompatible due to the action of self-incompatibility (S_x) alleles. The self-sterility may sometimes be suspended in the presence of pollen of a foreign species (*cf.* TORKA, 1951), a phenomenon previousley known in *Galium* (FAGERLIND, 1937).

In the present investigation it is, above all, the mechanism of incompatibility between *S. acaule* and *S. tuberosum* that is of interest. This mechanism is perhaps of essentially the same type as in the *Petunia axillaris* $\times$ *P. violacea* crosses investigated by MATHER (1943). If this is true, the pollen of *S. acaule* should carry self-compatibility alleles corresponding to the S_a genes of *P. axillaris*. In the style these alleles cannot inhibit S_x pollen from *S. tuberosum*, while styles containing S_x factors do inhibit S_a pollen-tubes.

In *Petunia* the F_1 hybrids could be divided into a self-fertile and a self-sterile group in accordance with the varying strength of the S_x factors, while in *Solanum* all the F_1 hybrids are self-fertile. When comparing these cases, it should be remembered that the *Petunia* species are diploid, and that the *Solanum* species concerned are polyploid. In the pollen-tubes of the *Solanum* hybrids there may be some more or less complicated interaction between S_x and S_a genes. Investigations on artificial amphidiploids of the *Petunia* hybrids would be interesting from this point of view.

A few seeds have been obtained from the cross between F_1 and *S. acaule* but the mating BC1a $\times$ *S. acaule* failed. Here the assumed S_x alleles of the female parent probably have a stronger inhibiting effect on S_a pollen than in F_1, since the former alleles are probably more effectively supported by their polygenic modifiers derived from *S. tuberosum* (*cf.* DARLINGTON and MATHER, 1949, pp. 254—259). The failure of this crossing indicates that the supposed S_x alleles are able to work in the cytoplasm of *S. acaule*.

In cases of one-way incompatibility between self-fertile and self-sterile species in *Solanum* and other genera the former are usually successful as pistillate parents, but exceptions are known. Chromosome doubled plants of the diploid, self-fertile species *S. polyadenium*, for instance, can only be crossed as male partners with *S. tuberosum* (SWAMINATHAN, 1951).

In the diploid species *S. phureja* and *S. Rybinii* self-incompatibility is probably determined by *S* alleles (*cf.* CARSON and HOWARD, 1942). Both species can be reciprocally crossed with *S. tuberosum* (STELZNER und LEHMANN, 1939; SWAMINATHAN, 1951). *S. phureja* is successful as female parent in crosses with *S. acaule* (KOOPMANS, 1951). The S_x genes of the former species do not inhibit the S_a genes, which according to this paper are supposed to exist in *S. acaule*. MATHER (1943), however, has pointed out that although related species have the *S* genes in common, they may create remarkably deviating incompatibility mechanisms.

Some cases of one-way incompatibility are also known among closely related self-sterile diploid *Solanum* species (*cf.* CHOUDHURI, 1944). It is also known among certain polyploid self-fertile species. The three autogamous species *S. acaule*, *S. antipoviczii* and *S. demissum* were all successfully crossed as female parents with *S. tuberosum*, while the reciprocal crosses are seldom successful and give male sterile hybrids (*cf.* p. 106), but these three self-fertile species, also when intercrossed, probably show one-way incompatibility. At any rate, in the literature on *Solanum* the combinations *S. acaule* × *S. antipoviczii* (PROPACH, 1938), *S. acaule* × *S. demissum* (PROPACH, 1937) and *S. antipoviczii* × *S. demissum* (KOOPMANS, 1951) are mentioned but not their reciprocals. At present, however, little is known about the genetical mechanism of inter-specific incompatibility within the section *Tuberarium*.

SUMMARY.

Hexaploid F_1 hybrids were obtained from crosses between artificial octoploids of *Solanum acaule* var. *subexinterruptum* BITT. and the variety Deodara of the tetraploid species *Solanum tuberosum* L. Reciprocal crosses proved impossible, since pollen-tubes of *S. acaule* are inhibited in the styles of *S. tuberosum*.

The F_1 hybrids were vigorous and rather fertile. Meiosis was fairly regular. Crosses with diploid, tetraploid and hexaploid species showed, that the functioning F_1 gametes usually have the triploid number of chromosomes.

Unlike *S. acaule*, the F_1 hybrids may be reciprocally crossed with *S. tuberosum*. F_1 crosses readily with *S. tuberosum* as male parent and gives a highly fertile pentaploid progeny. It is much more difficult to obtain seed from the reciprocal cross, and the likewise pentaploid

progeny thereby obtained is absolutely male sterile, and also has a somewhat reduced female fertility. The meiotical behaviour of the PMC's of the male sterile plants is abnormal. This male sterility is probably due to an interaction between the cytoplasm of *S. tuberosum* and one or more genes from *S. acaule*.

The hexaploid F_1 plants also give seeds although not often when used as pistillate parents in crosses with *S. acaule* as the male parent. This is not the case with the pentaploid plants obtained from backcrosses to *S. tuberosum*.

Through backcrosses with *S. tuberosum* as the recurrent parent, an increasing proportion of the *S. tuberosum* nucleus was introduced into the cytoplasm of *S. acaule*. This does not seem to deteriorate the fertility.

Some causes of sterility and incompatibility in the tuberous *Solanum* species are discussed.

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SUBARCTIC POLYPLOIDY

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I. INTRODUCTION.

DURING the past few decades many inquiries have been made into the question of the significance of polyploidy for the geographical and ecological distribution of plants as well as for their evolutionary history. Certainly, panalloploidy has played a great role in the creation of new genera and even families in the past, and hemialloploidy as well as autoploidy are assumed to have affected the formation of at least one third of the species of angiosperms known at present (*cf.* LÖVE and LÖVE, 1949; STEBBINS, 1950).

It was observed already by TÄCKHOLM (1922) that polyploids and diploids usually inhabit ecologically and climatically somewhat different areas with the former ones commonly extending farther northwards than the latter ones. HAGERUP (1931), who had made studies on the distribution of the diploids and polyploids of many genera, set forth the hypothesis that the polyploids might be better adapted for more extreme climates than are the diploids. This hypothesis might seem to have been verified, at least for Europe, as it has been clearly demonstrated that the frequency of polyploids within the whole floras, as well as within different orders and families and life form groups, increases significantly with an increase in latitude (for references, *cf.* LÖVE and LÖVE, 1949, 1952). It has also been assumed that the hypothesis might fit for different altitudes (MÜNTZING, 1936; SOKOLOVSKAJA and STRELKOVA, 1938, 1939, 1940), although this is denied by GUSTAFSSON (1947, 1948). According to some authors (*cf.* LÖVE and LÖVE, 1949), the rate of polyploids should mirror not only the extremeness of the climatic conditions but the severeness of the edaphic conditions as well, and the role of polyploidy in the creation of weeds is still under discussion (*cf.* FELFÖLDY, 1947, 1948; GUSTAFSSON, 1948; HEISER and WHITAKER, 1948; SCOTT, 1950). The same holds true also for the hypothesis on the wider distribution areas of the polyploids (*cf.* STEBBINS, 1950), as well as for some other more or less weakly supported suggestions regarding the geobotanical differences of diploids and polyploids.

It has been emphasized by STEBBINS (1950) that the increase in the frequency of polyploids with an increase in latitude as demonstrated

in Europe might be caused by the fact that polyploids seem to be ideally suited to the colonization of areas newly opened to plants. Certainly, this may be true for the increase observed from the unglaciated areas in southern and central Europe up to central and northern Scandinavia, which was completely covered by the Pleistocene glaciers. The highest rates of polyploids are, however, known from some subarctic and arctic islands, which are regarded to have been isolated from other countries since before the last glaciation, to say the least, so that their present floras are mainly made up of species which survived the glaciation on icefree refugia as well as of species incidentally introduced by man and later naturalized. It is, therefore, not likely that the high frequency of polyploids on these islands should be caused by the same type of tolerance as those suggested by STEBBINS (1950) for the other type of areas, and it may easily be found that the most probable hypothesis explaining this phenomenon is possibly the suggestion of the greater hardiness of polyploids than of diploids under northern conditions made by HAGERUP (1931), TISCHLER (1935), MÜNTZING (1936), and LÖVE and LÖVE (1943), although it is perhaps not the only plausible one.

It is of interest to test the validity of some of the hypotheses of the geobotanical significance of polyploidy on the flora of subarctic islands with a high frequency of glacial survivors. The present paper is intended to give such an evaluation for the subarctic island of Iceland, which, according to geologists and biologists (for references, *cf.* LÖVE, 1951; LÖVE and LÖVE, 1951, 1953) must have been isolated from other countries by wide oceans at least since prior to the last glaciation or, most probably, since late Miocene.

Iceland has an indigenous flora consisting of 389 species of higher plants (*Hieracium* and *Taraxacum* excluded), most if not all of which have survived the glaciation in the country, as well as of 122 species which have been naturalized during the past 1100 years. The chromosome numbers of all these species have now been determined on Icelandic material (LÖVE and LÖVE, unpubl.), so that the criticism of using numbers counted on foreign material for calculations such as the present one (*cf.* ARWIDSSON, 1938) is not justified in this special case.

II. POLYPLOIDY IN INDIGENOUS PLANTS.

In all previous calculations the indigenous as well as the naturalized elements of the Icelandic flora have been counted together for the

frequencies of diploids and polyploids (*cf.* LÖVE and LÖVE, 1949). The total flora of Norway is known to have about 58 % polyploids, that of Spitsbergen includes about 74 % of polyploids, while 65,8 % of the 511 indigenous and naturalized Icelandic angiosperms are characterized by polyploid chromosome numbers. The number given above for Spitsbergen does not include naturalized plants, and at least the great majority of the Norwegian species included in the calculations certainly belong to the groups of plants which invaded Scandinavia from the south and east after the last glaciation (*cf.* HULTÉN, 1950). Therefore, if the flora of Iceland is to be compared with the floras of these countries, the number giving the most reliable account of the conditions prevalent when the present flora was formed is arrived at if only the really indigenous plants are included. Within the 389 indigenous species there are 71,2 % polyploids, or only a slightly lower rate than that given for Spitsbergen. The extremely severe conditions of the Pleistocene, which made the selection of the Icelandic as well as the Spitsbergian flora, must have been arctic. The precipitation might, however, have been somewhat higher in Iceland than in Spitsbergen and other still arctic regions (*cf.* DAHL, 1946), so that a relatively higher number of tender diploids may have been protected by snowcover in the winter. This suggestion is strongly borne out by the fact that within the flora of the high arctic islands of Franz Joseph Land there is as high a rate of polyploids as 85 % (*cf.* LÖVE and LÖVE, 1952).

It is a well-known fact that the frequency of polyploids is always higher within the monocotyledons than within the dicotyledons (*cf.* LÖVE and LÖVE, 1943, 1949). In Iceland and Spitsbergen this is equally true, but while this number is definitely higher for the monocotyledons of Spitsbergen (95 %) than for those of Iceland (90 %), there is a much smaller difference between the dicotyledons, as their rate is 61,4 % and 60,3 %, respectively.

Based on the distribution areas of the species outside Iceland, the indigenous flora of the country is classifiable into several major groups. The *circumpolar element*, or the species with a complete or practically complete circumpolar distribution, comprises as many as 40,3 % of the indigenous plants; the *eastern element*, or the species with an Eurasiatic distribution area reaching their westernmost localities in Iceland, includes 30,6 % of the flora; the *bis-Atlantic element*, or the species growing on both sides of the Atlantic but not far inland east and west, includes 17,8 % of the flora; the *western element*, or the American species usually not reaching east of Iceland, accounts for 7,2 % of the plants,

while the rest of the flora, or 4,1 % of the species, is known as the *endemic element* of Iceland. As this last element also ought to include almost all the microspecies of *Hieracium* and *Taraxacum* it is, in fact, considerably larger than here shown, but as the agamospecies of just these genera are by no means comparable with the species of other groups, it is regarded as being most plausible to exclude them from these calculations.

Calculations on the frequencies of polyploids within the elements of the Icelandic flora show that 71,3 % of the species (92,0 % of the monocotyledons and 53,0 % of the dicotyledons) of the *circumpolar element* are polyploids; within the *eastern element* there are 68,9 % polyploids (86,2 % within the monocotyledons and 63,3 % of the dicotyledons); the *bis-Atlantic element* includes 72,5 % polyploids (86,2 % of the monocotyledons and 62,5 % of the dicotyledons); in the *western element* there are 67,1 % polyploids (87,5 % of the monocotyledons and 60,0 % of the dicotyledons); while the *endemic element* shows as high a frequency as 87,5 % polyploids (100 % of the monocotyledons and 81,8 % of the dicotyledons).

Of these numbers the ones for the endemic element are statistically different from the rest. It is, however, worth while here to point out that the endemic element is a small one and its members belong to species or groups classifiable into all the different non-endemic elements. Of course, it is not impossible that the polyploids might have some greater tendency of forming endemisms in arctic and subarctic regions, although the present writer does not think the present material supports such a suggestion. He, therefore, regards this significant difference only as a mere coincidence.

It is, however, certainly not a coincidence that the small differences observed between the rates of polyploids of the other Icelandic flora elements are of no statistical value. As already pointed out by STEBBINS (1950), there is no universal tendency for polyploids to have wider geographical ranges than their diploid ancestors, although this has been claimed to be the case by WESTERGAARD (1944), SÖRENSEN and WESTERGAARD (1946), FELFÖLDY (1947), DE SOÓ (1947), and others. The highest rate of polyploids of the Icelandic flora is met with in the bis-Atlantic element characterized by the smallest total area of distribution, while the circumpolar element with the widest total area shows a slightly lower frequency of polyploids. Nevertheless, there is no doubt that within some genera the polyploids tend to have a considerably wider area of distribution than the diploids, although this is by far a general rule.

III. POLYPLOIDY IN ALIEN PLANTS.

As high a frequency as 23,5 % of the Icelandic flora consists of species which have been introduced and naturalized in the country sometimes during its 1100 years of settlement. Some of these species are noxious garden weeds, others have evidently escaped from cultivation, while a great part of this group is more or less anthropochorous plants which seem to prefer roadsides and soils near houses or farms.

Within the total element of alien plants in the Icelandic flora there are only 48,3 % polyploids, or a significantly lower number than that of the total indigenous flora. Only 17 of the naturalized species are monocotyledons, but also their frequency of polyploids, or 76,5 %, is definitely lower than that of the native elements, and this is still more true for the 43,8 % polyploids of the dicotyledons.

Although the present investigation is the first study including all the species of naturalized plants known to occur within a region, statistical inquiries into the significance of polyploidy for extraneous species extending their range, weeds, or ruderal plants, have been made by several scientists in Europe as well as America.

TISCHLER (1937) pointed out that on the Halligen Isles in the North Sea the naturalized species originally introduced by man show a considerably higher frequency of polyploids than does the natural element of the flora, while the reverse is true for non-established weeds. This observation might seem to have been borne out by the studies made by HERMANN (1947) on the flora of fallow fields, by MÖLLER (1949) on the vegetation of ruins and refuse heaps, by CHRISTIANSEN (1949) on the societies of refuse heaps, as well as by FELFÖLDY (1947) on Hungarian ruderal communities. It might also seem to be confirmed by the results obtained by GUSTAFSSON (1948), who found that hemerophilous species in the Ladoga area show a significantly higher rate of polyploids than do the hemerophobous elements of the same region.

Two recent American investigations are of special interest in this connection. In a very stimulating paper on Californian weeds, HEISER and WHITAKER (1948) tried to reveal the connection between polyploidy and weedy conditions of a number of species most of which are alien in that region. They arrived at the conclusion »that in general polyploidy is an unimportant factor in determining whether a particular species will become an effective weed». The frequency of polyploids within the 175 species studied was found to be about 49 % (about

65 % within the monocotyledons and 42 % of the dicotyledons). From this, the authors also assumed that this might be very close to the frequency of polyploids within the flora as a whole. The present writer believes from other reasons that the first conclusion is a very plausible one, although he is not inclined to endorse the latter view, as the frequency of polyploids in this subtropical region might be assumed not to be higher than that of the Mediterranean countries already known (*cf.* LÖVE and LÖVE, 1949).

The alien or extraneous plants of Indiana were studied from the cytological points of view by SCOTT (1950). This author clearly demonstrated that out of 172 species cytologically known 56,5 % are polyploids, or 42 % of the dicotyledons and 64 % of the monocotyledons. It was also shown that 50 % of the alien species reaching their northern limits in Indiana are polyploids, while 69 % of the species reaching their southern limits in the region have more than the diploid number of chromosomes. This certainly indicates that the hypothesis of the increased frequency of polyploids with an increase in latitude as arrived at in Europe also fits for the North American continent. Unfortunately, no estimate of the rate of polyploids within the indigenous flora of Indiana has yet been made, but it is most probably slightly lower than that of the extraneous species.

Although it might seem as if the American results as well as at least some of the results obtained by the European scientists support the assumption that polyploids should be better adapted for adverse conditions or for introduction into new areas than are the diploids, the Icelandic results do not bear out that idea. It is, however, worth while here to point out the similarities in the frequencies of polyploids within the extraneous elements of different regions. In the Icelandic flora, 43,8 % of the alien dicotyledons are polyploids, the number for the same group in Indiana and California is 42 %, and according to GUSTAFSSON (1948) the average for the weeds in Scandinavia, Canada, and Switzerland is 44 %, 40 %, and somewhat more than 40 % polyploids, respectively. The present writer favours the opinion that this is no coincidence but that the similarities in the frequencies of polyploids within the extraneous element of floras as widely different as those just referred to are caused mainly by the fact that most weeds have been carried by the white man from western Europe, where the frequency of polyploids within the natural flora is close to that of the alien elements of the countries studied (*cf.* LÖVE and LÖVE, 1949; TISCHLER, 1950; HASKELL, 1952). This conclusion does, of course, not

influence the possibility that within the alien and weedy elements differences in the reaction of diploids and polyploids might be met with under edaphically different conditions, as already indicated by the studies by FELFÖLDY (1947), HERMANN (1947), CHRISTIANSEN (1949), and MÖLLER (1949).

IV. POLYPLOIDY IN THE MOUNTAINS.

It was pointed out by MÜNTZING (1936) and demonstrated by SOKOLOVSKAJA and STRELKOVA (1938, 1939, 1940) that the rule of the increased frequency of polyploids with an increase in the extremeness in climate might fit not only for the latitudinal distribution of plants but also for their altitudinal range. In other words, the same changes in the rate of polyploids are assumed to be met with when climbing a mountain in a temperate region where the frequency of polyploids is relatively low on the lowland, as those observed when travelling towards the pole, the highest rates being expected in the Arctic and near the summit of the mountain. According to GUSTAFSSON (1947, 1948) this is, however, not correct, as the rate of polyploids studied by him from Scandinavia was claimed to be even lower in the mountains than in the lowland.

It is to be expected from the geobotanical points of view that the rule advocated by MÜNTZING (*l. c.*) should not fit well for the arctic or subarctic regions, where the rate of polyploids in the lowland is already a very high one, and where the conditions in the lowland are not very different from those of the highlands farther south. Nevertheless, it is of some interest to test the hypothesis on subarctic material, although it cannot be proved or disproved by aid of such material only.

In a good paper on the vegetation of Árskógsströnd in northern Iceland, DAVIDSSON (1946) gave a survey of the flora at different elevations from the coast up to the mountain summits at about 900 m. s. m. Below 100 m. s. m. there is a total of 190 species, 72 % of which are polyploids, while above the 600 m elevation there are only 67 species 66 % of which are polyploids. The slope faces east and south. The decrease in rate is statistically insignificant.

In the most excellent survey hitherto published of the vegetation of the central highland of Iceland, STEINDORSSON (1945) gives good lists of the floras of some mountains in the southern parts of the highland. The rate of polyploids as calculated by the present writer in the southern

lowland is about 70 %, while the flora of the southern slope of the mountain Lodmundur between 600 and 1010 m. s. m. counts only 42 species, 74 % of which are polyploids. Above 1000 m. s. m. on this southern slope there are only 12 species reported, 9 of which, or 75 %, are polyploids. Thus, the frequency of polyploids does not show significant differences on this mountain either.

From STEINDORSSON (*l. c.*) the list for the northern slope of the mountain Sáta in the same region was used for calculations of the rate of polyploids. There are only 25 species on the slope from 600 to 800 m. s. m., 80 % of them being polyploids. Above 800 m. s. m., however, there are 16 species with only 69 % of polyploids.

Based on the tables by STEINDORSSON (*l. c.*) the following calculations of the frequency of polyploids within some typical plant communities of the highland were made. A *Carex Bigelowii—Salix* mire at Laugar has 66,7 % polyploids, while a *Calamagrostis neglecta* mire margin at Arnardalur revealed a frequency of 77 %. A *Kobresia—Campanula rotundifolia* slope rich in grasses at Vídidalur has 82 % polyploids. At Kringilsárrani a typical snowpatch vegetation was classified as a *Taraxacum cfr. croceum—Ranunculus acris* association. It included 54 % of polyploids, while other snowpatch associations were found to be characterized by up to 64 % polyploids, which was typical for *Alchemilla—Salix—Anthoxanthum alpinum* snowpatches at Kýlingar. A *Salix—Kobresia* dwarf shrub heath at Arnardalur included 76 % polyploids, and a *Rhacomitrietum* heath at Hvannalindir showed 79 % polyploids. Three clayey flats sociologically analysed by STEINDORSSON (*l. c.*) showed 86 %, 90 %, and 100 % of polyploids, respectively, but *Salix herbacea— Bistorta vivipara* gravel flats had only 69 % of polyploids. A morain at Eyjabakkar was found to be covered by 37 species of angiosperms, 65 % of which were polyploids, and a typical tundra mire at Kringilsárrani was characterized by 69 % polyploids on the summits of the hummocks.

From the data given above it might seem as if the opinions set forth by GUSTAFSSON (1947, 1948) were amply verified. Nevertheless, the present writer does not think this material supports any criticisms of the hypothesis as it was set forth by HAGERUP (1931), TISCHLER (1935), and MÜNTZING (1936), as the differences between the lowland and highland in subarctic regions with a high frequency of polyploids are expected to be small and insignificant. From the few sociological data here analysed it is, perhaps, not easy to get a reliable correlation between the extremeness of the edaphic and climatic conditions and the

rates of polyploids. However, communities covered by light snow and rather tight vegetation seem to show practically the same frequency of polyploids as does the total flora of the island. The most extreme conditions, as, *e. g.*, those of the clayey flats, show the highest rate of polyploids, while the communities best protected against cold and solifluction, or the snowpatch associations, are found to show the lowest rate. This is just what could be expected from the point of view of the hypothesis, and not the reverse, as assumed by GUSTAFSSON (1948).

When discussing the rates of polyploids under different conditions in an arctic or subarctic region with a high frequency of polyploids, it has to be kept in mind that although the high-arctic monocotyledons are always polyploid, the most high-arctic and high-montane species of the dicotyledons are still diploids, so that the rate of polyploid dicotyledons will increase, theoretically, up to perhaps 70—80 %, and then decrease again under the most extreme conditions. This shows that the tolerances making it possible for plants to survive the severe climates are met with already in the diploid state and not created by polyploidy *per se*. However, as these tolerances are not wide enough in most genera to enable them to survive under the most extreme conditions, their species would not be able to reach the higher latitudes and altitudes were it not for the fact that the wider genetical variability after polyploidization may widen and strengthen these tolerances. Without this effect of polyploidy the floras of the most northern countries and the highest mountains would have been considerably more scarce than they are at present.

Although polyploidy certainly is a positive feature for species of high latitudes, high altitudes, as well as of different more or less extreme edaphic conditions, the species does not react against such conditions by creating polyploids. Such a Lamarckian view does not have the faintest support in any of the cases yet studied, as at least practically all the species of the Arctic are found to have a distribution area indicating a still wider area before the Pleistocene glaciations began. All but one of the Icelandic endemisms have, too, been formed by the way of genical isolation and selection only, as their closest relatives abroad are known to be characterized by the same chromosome number, and the only one left is an alloploid. In this connection it is worth while to point out that also the assumption made by GUSTAFSSON (1948) that »culture conditions contribute ... to the change of annual diploids into polyploid weeds» is without any geobotanical support, as all known weeds have some natural areas, although these are not al-

ways easily determinable at present (*cf.* HULTÉN, 1950). Even the cultivated plants, which without doubt owe their present distribution to mankind, were certainly not changed cytologically by the cultural conditions, but as they were found to be practical and positive man helped to conserve them by cultivation.

SUMMARY.

(1) The Icelandic flora consists of 511 indigenous and naturalized plants, 65,8 % of which are polyploids. Out of the 389 indigenous species, which survived the Pleistocene glaciations in the country, as many as 71,2 % are polyploids, or 90 % of the monocotyledons and 60,3 % of the dicotyledons. This is slightly lower than in the arctic flora of Spitsbergen.

(2) There are no significant differences in the rate of polyploids of the four major elements of the flora. The high frequency of polyploids in the endemic element is assumed to be a coincidence only.

(3) Only 48,3 % polyploids are in the 122 alien plants naturalized in the country. It is assumed that the frequency of polyploids within such elements is determined only by the frequency of polyploids within the floras they originated from, in this case northern and central Europe.

(4) The mountain flora in Iceland does not show higher frequencies of polyploids than does the lowland flora. This is assumed to be caused by the fact that the flora of the lowland already shows a rate of polyploidy close to the highest one obtainable.

(5) Most montane plant associations are found to be characterized by a rate of polyploids close to that of the country as a whole. The most extreme associations of the clayey flats, which are not protected against frost and solifluction, are found to have the highest frequency of polyploids, while the protected snowpatches revealed the lowest ones. This supports, in the view of the author the hypothesis of the greater tolerance of polyploids to extreme climates of high elevations even in the Arctic.

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PROGENY STUDIES IN THE HYBRID POA PRATENSIS x POA ALPINA

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SEED formation from *Poa pratensis* L. (coll.) and *Poa alpina* L. has, in recent years, been the subject of extensive studies. GUSTAFSSON (1946, 1947) has surveyed the work up to 1947. *Poa pratensis* is notable for the wide variation of its chromosome numbers (2*n* normally ranging from 50 to 100). Within the species apomictic, semiapomictic and sexual types occur. The apomictic types are most frequent. In these there is aposporous embryo-sac formation developing by mitotic division. The E. M. C. undergoes reduction division and usually forms a triad which can, at an early stage, be pushed aside by the aposporous cells; at times, however, it develops much earlier, and is fertilized. The egg nucleus in the aposporous embryo-sac is not normally fertilized, but this is possible, and then triploid-derived plants are formed. Haploids are also formed fairly frequently. The central nucleus must be fertilized before endosperm formation (MÜNTZING, 1933; ÅKERBERG, 1939, 1942; TINNEY, 1940).

In *Poa alpina* the chromosome number (2*n*) in natural types has varied from 14 to 57. In the sexual types the embryo-sac mother cell undergoes meiotic division, and four haploid macrospores are formed. In the apomictic types the macrospore mother cell nucleus divides mitotically. The egg cell divides early and usually an embryo is formed before pollination occurs. The central nucleus is fertilized all the time, and the endosperm is pentaploid (HÅKANSSON, 1943).

In crosses between apomictic *Poa pratensis* and *P. alpina, alpina* pollen has, in its main features of seed formation, the same effect as *pratensis* pollen, in which normal *pratensis* plants are obtained. Sometimes hybrids are formed, and these occur as often as the true crossing products between *pratensis* plants. Crosses between sexual *pratensis* and *alpina* give normal hybrids and can be easily produced.

A number of hybrids between *Poa pratensis* and *P. alpina* have now been studied. They have come from apomictic and sexual *pratensis,* and from *pratensis* plants with greatly varying chromosome numbers. Certain F_1 plants have been described earlier (ÅKERBERG, 1942). An

account will be given of the F_2—F_4 derivatives from these families. In their main points, the morphological descriptions relate to the development shown by the plants when planted out at Ultuna, Uppsala. The plants of different *Poa* species have been rather weakly developed owing to dry weather during the early summer.

EXPERIMENTAL RESULTS.

Hybrid plant A. G 251/2 from Poa prat. 702 × P. alp. G 44.
2n = 41 50 31

G 251/2, which is consequently a diploid hybrid, has already been described (ÅKERBERG, *l. c.*) as well as certain families derived from it. From nine F_2 families sown out, two developed after crossing again with *Poa pratensis,* four after crossing with *P. alpina,* two after isolation (= is), and one after free flowering (= sp). In F_2 the following chromosome numbers have been observed:

Fam. 1	G 251/2	× *Poa prat.*	2n = 35, 41, 73
2	»	× *Poa alp.*	2n = 35, 36, 38, 39, 40, 47
3	»	is	2n = 50
4	»	sp	2n = 68

As a result of morphological studies of the plants in the families 1—4, it can be stated that the hybrid plant G 251/2 has, in general, had sexual seed development. Investigations of the chromosome numbers of the plants show that meiosis must be very irregular, with gametes of greatly varying chromosome composition as a result. Certain chromosome numbers indicate that unreduced female gametes — probably of aposporous derivation — are in some cases functioning. That this is a possibility is also shown by the embryological investigations.

Six plants, all derived from back crossing with *alpina,* have been followed to F_3 and F_4. A special group contains three of the plants, all alike and identical with the father plant G 44. The progeny from these — four families were investigated — were plants constant in all respects, and the chromosome numbers from six plants, two from each of the three mother plants, were 31 — or identical with the father plant. Earlier it was assumed that the plants had been formed by male parthenogenesis. The result from the F_3 investigation does not in any way contradict this assumption. Embryological investigation of the material has not been carried out.

The remaining three families have also been of interest. The first, Ä 51, is derived from a mother plant with $2n = 36$. The progeny from this (F_3) showed strong morphological variation with many weak plants. F_4 has been grown from one of these plants. This family was also variable and some of the plants were of low vitality. The chromosome numbers have been investigated in two of the plants (twins), and were 88 and 87, respectively. Embryological investigation of the plants showed E. M. C's by meiotic division, but also revealed signs of degeneration. Aposporous cells were observed early, before the E. M. C's had begun division. These aposporous cells lie beside the E. M. C's or in the chalaza region. The chromosome numbers 88 and 87 point to successive fertilization from F_2—F_4 of unreduced E. M. C's (F_2 $36 + 18 = 54$, F_3 $54 + 27 = 81$). The diffference between the expected chromosome number (81) and those found (87—88) can be due to the occurrence of pollen with chromosome numbers other than 18 and 27, respectively.

The second of the three families, Ä 52, was derived from a plant in F_2 with $2n = 35$. F_3, sown out twice, gave morphologically similar plants. This also occurred in F_4, where four plants had $2n = 35$, thus confirming to the mother plant in F_2. All the plants were small and weak. Aposporous cells were frequently seen in the chalaza region of the nucellus in all the embryologically investigated plants. The E. M. C. was sometimes degenerated. Pollen formation was also abnormal and pollen plasmodia appeared. Pollen for an investigation of pollen quality could not be obtained. There was consequently from the cross (*Poa prat.* $2n = 50 \times$ *Poa alp.* $2n = 31) \times$ *Poa alp.* $2n = 31$ [(aposporous × diplosporous)] a plant in F_2 ($2n = 35$) with mainly aposporous development, which was checked in F_3 and F_4.

The third F_4 family, Ä 53, is derived from an F_2 plant in which the chromosomes were not investigated. In F_3 there was only one plant. In the F_4 family $2n$ was determined in five plants, giving the values 54, 55, 55, 55, and 56. These are all alike. The plants in this family are strong, forming runners and reminiscent of *Poa pratensis*. A morphological variation was to some extent present but it could not be decided with certainty whether or not it was only a modification. The aposporous cells appeared early and the E. M. C. seemed to have degenerated. The mother plants of Ä 53 seem also to have had aposporous seed formation and to have probably come from fertilization of an unreduced E. M. C.

Hybrid plant B 1. Ä 62/7 from Poa prat. 701 × Poa alp. G 137.
2n = 45 50 37

Ä 62/7 is, like G 251/2, a diploid hybrid obtained from the parent plants with, in the main, apomictic seed formation. Two F_2 families have been studied, of which one after back crossing with the father plant G 135. The plants in this family were weak, 2*n* for two plants investigated being 48—50. In the second family the plants showed variations in different features — with chromosome numbers (2*n*) in two of them of 65 and 75. These plants have probably come from fertilization of the unreduced E. M. C. Both plants have been studied to F_3. From the one with $2n = 65$ a family (Ä 54) of twelve plants has been sown out. These vary greatly in, for example, height and circumference of tuft. The chromosome numbers of eight plants were 93, 95, 96, 98, 99, 108, 108, and 112. *All* of these plants seem to be derived from fertilization of unreduced egg cells. A small number of flowers of the F_3 plants have been studied embryologically. Aposporous cells have been observed and, to a certain extent, degeneration of the E. M. C. From the E. M. C. a triad has sometimes been seen.

38 plants (Ä 55, Ä 56) have been studied from the F_2 plant with $2n = 75$. Distinct differences in vitality, height, and circumference of tuft have been observed. A good many plants were weak. In ten plants the chromosome number varied between 60 and 78 (60, 65, 71, 71, 72, 73, 73, 74, 74, and 78). In an eleventh plant 2*n* was 16. The chromosome numbers also speak for a predominant sexual seed formation in the mother plant. In embryological investigations aposporous cells have been observed, and in some cases degeneration of the E. M. C.

Of very great interest is the subhaploid plant with $2n = 16$. The plant is morphologically very like *Poa trivialis*, agreeing with another subhaploid plant from *Poa pratensis*, found by KIELLANDER (1942). The plant is quite sterile, no viable seeds being developed. It otherwise possesses good vitality and is able to live a number of years.

Hybrid plant B 2. Ä 62/9 from Poa prat. 701 × Poa alp. G 135.
2n = 69 50 37

This plant, Ä 62/9, is derived from the same cross as the previous Ä 62/7. The difference is that Ä 62/9 is a triploid hybrid, obtained by fertilization of an unreduced egg cell of the mother plant *Poa pratensis*. A number of F_2 and F_3 plants have been studied. Some of the F_2 plants have already been described (ÅKERBERG, 1942). There were four F_2 families developed from isolated panicles and free flowering, and after

back crossing with the father plant G 135. All the families showed evident segregation. From one F_2 family, obtained after free flowering, six F_3 families were sown out, descended from a similar number of free-flowering F_2 plants. The following chromosome numbers have been observed:

Family (F_3)	$2n$
Ä 57	62, 64, 72
Ä 58	67, 72
Ä 59	50, 50, 51, 52
Ä 501	52, 112, 112, 113, 117, 122
Ä 502	50, 53, 53, 54, 54, 54, 54, 54, 54, 54, 54, 54, 55, 96, 98, 104, 108
Ä 503	63, 72

Of these families Ä 57, 58 and 503 appeared approximately the same. The plants were small and weak, and a number died at an early stage. The morphology as well as the variation in chromosome numbers indicate that they were sexually reproduced from the mother plant.

The plants in Ä 59 have also shown low vitality but they have been fairly uniform. The chromosome numbers further indicate apomictic seed formation. The same will probably also hold good for the mother plant of Ä 502, in which family most of the plants have been strongly developed. The plants with the high chromosome numbers, varying between 96 and 108, will probably be derived through a fertilization of unreduced embryo-sacs, in which the pollen seems to have had a high chromosome number. Ä 501 has consistently shown great variation in vegetative development, indicating sexual seed development in the mother plant. The chromosome numbers, which support such a conclusion, are remarkably high. It is not certain when and how these developed.

In the families Ä 57 to Ä 503 no embryological studies have been made.

Hybrid plant C 1. Ä 733 pl. 1. 2. 4. from Poa prat. 709 × Poa alp. G 261.
$2n = 70, 66, 70$ 90 50

One hybrid plant, No. 4 ($2n = 70$), has been investigated in F_2 and F_3. Earlier investigations on the parent plants and on a great number of progeny plants have shown that *Poa pratensis* plant 709 has predominantly sexual reproduction, and that *Poa alpina* plant G 261 is apomictic. The F_2 family is clearly variable in its different morphological characteristics. From three free-flowering F_2 plants,

three F_3 families have been produced with the following chromosome numbers:

F_3 Family Ä 504 $2n = 94, 94, 98, 104, 108, 110, 112, 115, 115, 119$
Ä 505 $2n = 75$
Ä 506 $2n = 101, 108$

In the three families Ä 504—506 the plants were low and small, and many of them were very weak. The stolons were missing and the tufts densely packed. Most of the plants flowered badly. Only a small number of embryological investigations were made. In the family Ä 506 it could be seen that the number of aposporous cells were small. Embryo-sacs with three megaspores were observed.

It is considered that the mother plant of the families Ä 504 and Ä 506 was developed by fertilization of unreduced egg cells. It cannot be proved whether they are of aposporous or diplosporous origin. The former seems to be most likely. But, on the other hand, it can be said that the mother plant Ä 709 was strongly sexual, although earlier observations (ÅKERBERG, 1942) show that aposporous cells, at times, appeared.

Hybrid plant C 2. Ä 734 pl. 1 from Poa prat. 709 × Poa alp. G 261
2n = 66 90 50

The plant is a sister plant to the previous F_1 family Ä 733, and in a similar manner has developed by fertilization of a reduced egg cell in the mother plant. One F_2 and three F_3 families have been studied, all coming from a free flowering of the respective mother plants. The chromosome numbers and embryology have only been investigated in the F_3.

F_3 Family Ä 507 $2n = 56$
Ä 508 $2n = 56, 72$
Ä 509 $2n = 49, 50, 50$

The plants from the families Ä 507 and 508 have grown and behaved in about the same way as the progeny of Ä 733 pl. 4. Two of the plants investigated had, however, rather low chromosome numbers. Ä 509 is a completely divergent type: it is uniform and has strong high plants with rich stolon formation. Morphologically, it seems to be pure *pratensis*, and it is feared that it may have come from a mixing of some kind. However, in earlier investigations material of this type has been found.

Hybrid plant D 1. Ä 629 pl. 7 from Poa prat. 746 × Poa alp. G 121.
2n = 95 80 not known

The mother plant of this hybrid, 746, has been previously described as a high grade apomictic type with well formed aposporous cells. The hybrid is considered to be derived from fertilization of an unreduced egg cell. (The chromosome number of the father plant, which is not known for certain, should be between 35 and 40.) The F_2 family has been sown out twice, both times after free flowering in the F_1. A rather clear segregation was apparent in F_2. Four F_3 families have been studied, and were developed after isolation and free flowering of the two F_2 plants.

F_3 Family Ä 510 $2n = 102$
Ä 511 $2n = 98, 103, 104, 144$
Ä 512 $2n = 102, 114$
Ä 513 $2n = 100, 101, 102, 105, 106, 107$

The plants in these four F_3 families have been evenly and strongly developed. Only a few plants died after planting (91 % were living after two years). It has been difficult to determine whether the plants are morphologically alike. The chromosome count does not give reliable information, as the high numbers prevent accurate reckoning. One of the plants in family Ä 511 has apparently come from fertilization of an unreduced egg cell. The others could have come from aposporous, followed by parthenogenetic, embryo-sac development. This can also be seen from the good vegetative growth in all plants.

Hybrid plant D 2. Ä 269 pl. 8 from Poa prat. 746 × Poa alp. G 261.
2n = 102 80 not known

This hybrid is also a plant of the same origin as the previous Ä 629 pl. 7. F_2 has been sown out in three families, one after free flowering and two after isolated panicles. The F_2 plants have been rather similar. Five F_3 families, derived from four different F_2 plants, have been examined and have given the following chromosome numbers:

F_3 Family Ä 514 $2n = 103, 105, 106, 106$
Ä 515 $2n = 103, 108$
Ä 516 $2n = —$
Ä 517 $2n = 100, 100$
Ä 518 $2n = 103, 104$

The plants in these F_3 families were clearly divergent from the F_3 plants from Ä 629 pl. 7. They were strongly developed, tightly packed

with dark stiff leaves, and without stolons. Clear morphological differences between the plants could not be demonstrated, and the chromosome count showed no variation great enough to be used for identification. We have probably here a hybrid plant that already in the F_1 had mainly apomictic seed formation. Embryological investigation has not been carried out in the F_3 plants of the hybrid Ä 629 pl. 7 and 8. The pollen was well developed and the pollen grains were strikingly large.

DISCUSSION.

Naturally and artificially developed hybrids between Poa pratensis and Poa alpina. — From the results of artificial crosses between *Poa pratensis* and *P. alpina* it is considered that hybridization of the species when they grow together in natural conditions might be expected. Different times of flowering and mainly apomictic seed formation of the parent species lessen, however, the chances of such hybridization. Hybrids from nature are, however, well known and have been described as *Poa herjedalica* (SMITH, 1920; and others). Recently, these hybrids have been the subject of a comprehensive investigation (NYGREN, 1950). All hybrid plants found in the mountains have been viviparous. NANNFELDT (1937) described, however, a non-viviparous plant from Uppsala. In NYGREN's material the chromosome numbers ($2n$) varied from 47 to 80. Apospory could be established in many of the plants investigated embryologically. Only one check plant had no aposporous cells. Here mitotic division in the E. M. C. was observed. NYGREN assumes that the hybrid plants found formed segregation products in the F_2 or later generations. These and the results as a whole from NYGREN's investigations agree extremely well with what would be expected from the work on artificially produced hybrids. In these greatly varying chromosome numbers are found and from them, as a rule, sexual F_1 plants undergo in F_2 and later generations reproduction of the apomictic type.

However, it is evident that practically all natural hybrids are viviparous. This is certainly related, firstly, to characters of the parent plants — *Poa alpina* (and, in some cases, also *Poa pratensis* subsp. *alpigena*) is viviparous within the area where *Poa herjedalica* is found. On the other hand, one must ask why non-viviparous hybrids are not found more frequently. The question can not be answered with certainty. The different flowering times are perhaps more important where non-viviparous forms of parent plants grow together. It is widely believed that naturally developed reproduction products which have the ability to

keep themselves back, come so near to their parents in their characters that they cannot be distinguished. In the material described above a product, which had practically completely reverted to *Poa pratensis*, has arisen in the F_3—F_4 stage. From hybrids between *Poa pratensis* and *P. ampla*, and between *P. pratensis* and *P. scabrella* the offspring behave in the same way (CLAUSEN, GRUN, NYGREN, and NOBS, 1951). These authors say that »as many as 10—15 % of the offspring of sexually reproducing hybrids of *pratensis* with other species may within two or three generations converge toward *pratensis*».

Inheritance of apomixis in Poa. — MÜNTZING (1940) seems to have been the first to discuss, on the basis of experimental studies, the genetic development of apomixis in *Poa*. He said that »apomixis in *Poa* is due to a rather delicate balance». This statement has been strongly verified in subsequent investigations. It seems that one can distinguish different phases in this development, phases which correspond to those that have been described for apomixis in *Parthenium* (POWERS, 1945). The phases can be outlined as follows: (1) The production of aposporous cells with capacity for E. M. C. formation or of diplospory. (2) The ability of parthenogenetic development of the egg cell. It is thought that the fertilization of the egg cell, irrespective of whether the chromosome number be n or $2n$, is dependent on the time for E. M. C. development in relation to fertilization. (3) The capacity of seed formation without fertilization of the egg cells both with and without fertilization of the endosperm nucleus.

What is stated here regarding apomictic development is well illustrated in the segregation results from the hybrid *Poa pratensis* × *P. alpina*. These can best be shown as follows:

Parents: *Poa pratensis* — Aposporous, pseudogamous.
Poa alpina — Diplosporous, pseudogamous.
F_1 — Mainly sexual.
F_2 — Reproduction products according to F_3—F_4 studies.

(a) Sexual plants — fertilization of reduced egg cells, *e. g.* families Ä 55, Ä 56.

(b) Sexual plants — fertilization of unreduced egg cells, *e. g.* family Ä 54. Egg cell from E. M. C. by aposporous development.

(c) Aposporous plants with parthenogenesis, *e. g.* Ä 52.

In addition haplo-parthenogenesis and possibly male parthenogenesis have occurred.

In the embryological studies diplospory has not been observed in any of the F_3—F_4 plants. The number of plants investigated has, however, not been very great. It is clear, in any case, that diplospory is a more complicated reproduction product — this is also seen from NYGREN's (1950) naturally hybridized plants.

Reproduction in F_1—F_4 for the hybrids *Poa pratensis* × *P. alpina* has many parallels with other *Poa* hybrids, *Poa ampla* × *P. pratensis*, *Poa scabrella* × *P. pratensis*, *etc.*, investigated by CLAUSEN, KECK and HIESEY (1947), and with the offspring from crosses in certain *Calamagrostis* species (NYGREN, 1951). Here, there was obtained a sexual F_3 with the necessity for the combination of the parent properties. In the F_2 apomictic products are formed. This necessity by species hybridization, of the breaking of apomixis and the later finding of it again in new types, gives good possibilities in the breeding work with *Poa*. It makes possible the use of new genome material from other species, which afterwards can be introduced by apomictic seed formation.

In the cited work of MÜNTZING (1940) there is also a discussion on the extent *Poa pratensis* is developed by hybridizing. MÜNTZING found that autopolyploidy was more apparent in *Poa alpina* than in *Poa pratensis*. The latter consequently should have more of a hybrid character. The strong hybrid character of *Poa pratensis* is also pointed out by CLAUSEN, GRUN, NYGREN, and NOBS (1951). The subhaploid *Poa trivialis*-like plants described by KIELLANDER (1942) and in this paper, indicate that *trivialis* should be one of the original species of *pratensis*. Here material is needed for complete proof, but both findings must constitute an important basis for wider investigations on the derivation of the complex chromosome set of *Poa pratensis*.

SUMMARY.

From the cross between *Poa pratensis* L. (coll.) and *P. alpina* L. nine hybrid plants have been studied, five obtained from apomictic types of the species, which have respectively aposporous and diplosporous embryo-sac formation, and four after a cross between sexual *pratensis* and apomictic *alpina*.

A. *Apomictic Poa pratensis* × *apomictic P. alpina.*

Of the five hybrid plants two were diploid hybrids ($2n = 41$ and 45) and three triploid hybrids ($2n = 69$, 95 and 102).

To judge from the characters of the F_2 plants (morphology, embryology and chromosome number) four of the five plants must have had mainly sexual seed formation. The fifth plant ($2n = 102$) seems to have a predominantly apomictic seed development.

In F_2 there was a segregation in different characters, of which the heredity has been controlled in 21 F_3 families and 3 F_4 families. In regard to the seed formation in F_2 the following types have been obtained:

(1) Fertilization of reduced egg cells (5 F_2 plants). Aposporous cells have been observed in some F_3 plants. In the progeny of one of the F_2 plants a subhaploid plant with $2n = 16$ was found. It was quite similar to *Poa trivialis*.

(2) Fertilization of unreduced egg cells. The unreduced egg cells have arisen from aposporous cells (2 F_2 plants). Hyperaberrants are formed with the chromosome number so far raised to $2n = 144$.

(3) Development of the unreduced egg cell without its being fertilized (apospory followed by parthenogenesis, 11 F_2 plants, of which two were rather unsure).

(4) Male parthenogenesis (3 F_2 plants, all from the same F_1 plant).

The number of families within the four groups is of course no expression in figures of the segregation. To be able to state this the zygote lethality must also be noted — it may be especially great in plants belonging to group (1). The segregation of the seed formation seems also to be different in different hybrid plants.

In the embryological examination of F_1 and F_3 plants no case of diplospory has been observed.

B. *Sexual Poa pratensis × apomictic P. alpina.*

All the four hybrid plants were hybrids and they showed a very great segregation in F_2. The same is the case in five of the six F_3 families studied (the sixth family is too unsure to be discussed). One would have expected that the chromosome numbers of the F_3 family would be just about the same as in the hybrid plants (where the number varied between 66 and 70). Two of the families seem to have been developed from F_2 plants, arising from fertilization of unreduced egg cells. It may be that gamete lethality gives an increased chance for the new aposporous cells, which are developed in these hybrid plants. In the mother-plant of *Poa pratensis* a tendency to apospory has also been shown.

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NUCLEAR FRAGMENTATION—A NORMAL FEATURE OF THE MITOTIC CYCLE OF LYMPHOSARCOMA CELLS

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NUCLEAR fragmentation which has often been described by cytopathologists has been variously interpreted as to its mechanism. The tendency has been to regard the fragmentation, undoubtedly occurring in many cases, as due to spindle disturbances during mitosis. As is well known, such mitotic disturbances may give rise to several small telophase nuclei at each pole instead of the regular formation of two daughter nuclei. Thus, among the various nuclear abnormalities induced by c-mitotic substances it is almost the rule that concentrations close to the c-mitotic threshold of any such substance may partially inhibit spindle function, which will result in the formation of abnormal spindles and consequent abnormal distribution of the daughter chromosomes, multinuclear or multilobate telophases being frequent.

On the other hand, it was recently shown (LEVAN and TJIO, 1951) that certain pathological conditions of a tissue may lead to the artifical fragmentation by bursting of resting nuclei, sometimes entire tissues being made up of cells containing numerous nuclear fragments. This condition is a purely pathological phenomenon preceding the eventual destruction of the tissue.

Cancer tissues are often characterized by the occurrence of multinucleate cells and cells with lobated nuclei. One of the ascites tumours studied by the present authors has turned out to be particularly interesting in this respect, *viz.* the mouse lymphosarcoma 6C3HED. This was established as an ascites tumour by KLEIN working at the Institute for Cancer Research during the fall of 1950. At that time about 10 % of the 6C3HED cells were multinucleate or had multilobated nuclei. Samples of the tumour analysed during 1951 showed a frequency of abnormal cells of about the same size order (Table 1). All the samples taken during 1952, however, showed a significantly higher frequency

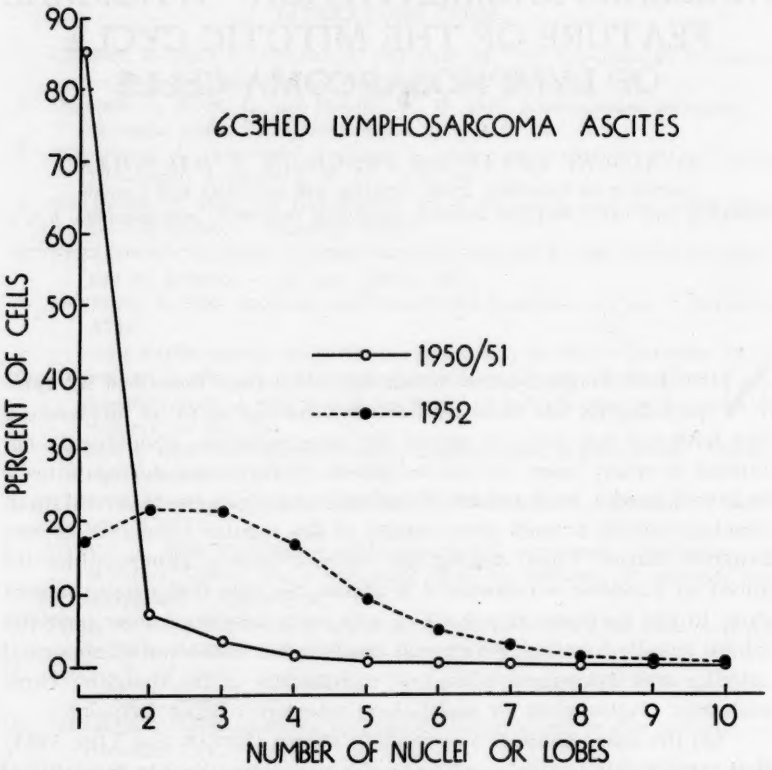

Fig. 1. Graph of the frequency of cells with micronuclei and nuclear lobes in the material from 1950/51 and from 1952.

of abnormal cells (50—99 %), at the same time the abnormalities were much more extreme than before.

Some data on the frequency of these abnormalities are given in Table 1. Samples of 100, or in some cases more, cells have been analysed as to the frequency of mononucleate, multinucleate, or lobated nuclear condition. Cells with pycnotic nuclei are given as a separate fraction since they often contain a high number of nuclear fragments of a type readily distinguishable. Finally the mitotic index was determined for 500 non-pycnotic cells of each sample.

The table and the graph of Fig. 1 show a striking difference in the frequency of multinucleate cells between 1950—51 on the one hand, and 1952 on the other. No certain difference between the various 1952

TABLE 1. ***The frequency of cells with micronuclei or nuclear lobes in the lymphosarcoma 6C3HED.***

Year	Generation No.		Days after inoculation	Percentage of cells with the following number of nuclei or nuclear lobes											% pycnotic cells	% mitoses
	Home	Foreign		1	2	3	4	5	6	7	8	9	10	>10		
1950/51	*	—	5	88,0	6,4	2,8	0,8	—	—	—	—	—	—	—	2,0	—
	4	—	9	88,7	7,3	2,4	0,7	—	—	—	—	—	—	—	1,0	1,4
	14	—	11	76,4	11,2	5,6	3,6	1,6	0,8	—	—	0,4	—	—	0,4	0,6
	14	—	11	90,4	4,4	2,8	1,2	0,4	0,4	0,4	—	—	—	—	0	—
1952	55	—	6	15	14	19	18	13	9	6	1	—	—	3	2	—
	55	1	6	13,2	18	22	18,8	10,4	7,2	4,2	2,4	—	0,4	0,4	3,2	3,0
	56	—	6	15	27	21	12	9	10	2	1	1	—	—	2	2,4
	55	1	8	25	26	24	13	7	—	3	—	—	—	—	2	2,8
	56	—	8	50	16	12	10	8	3	—	—	—	—	1	0	2,0
	55	1	9	17	28	14	9	2	1	—	—	—	—	—	29	1,2
	55	1	9	1	11	25	18	12	4	2	1	—	—	—	26	1,8
	56	—	9	15	25	23	20	9	4	1	1	—	—	—	2	1,0
	55	1	11	17	28	28	18	7	2	—	—	—	—	—	0	1,8
	56	—	11	21	28	22	16	11	1	—	1	—	—	—	0	0,8
	55	2	7	8	21	29	22	13	4	2	—	—	—	—	1	1,2
	55	3	7	9	22	19	16	10	7	3	2	1	—	—	11	2,2
Total 1950-1951				85,8	7,3	3,3	1,5	0,5	0,3	0,1	—	0,1	—	—	0,9	—
1952				16,8	21,6	21,5	16,2	9,4	4,7	2,2	1,0	0,2	0,1	0,4	6,1	—

* Third transfer after the establishment of 6C3HED as ascites.

samples is seen. The main appearance of the resting nuclei seems to be the same whether the tumour is grown without interruption in the home strain (C3H/St, where it is 100 % lethal) or for occasional side-passages in a foreign strain (Swiss mice, where it always regresses). Neither does the age of the tumour counted from the day of inoculation appear significant. It should be noted, though, that the three cases in which a high frequency of pycnotic disintegrating nuclei were found were all from tumours grown in hosts of a genotype distinct from that of the tumour.

Dr. G. KLEIN has very kindly examined nuclear conditions in 6C3HED lymphosarcoma, maintained at his laboratory in Sweden, our subline of the tumour having been derived from his stock 60 transfer generations ago. We are indebted to him for the following counts, wherein each value is based on 500 cells:

Date of sampling		Days after inoculation	Percent abnormal cells		Percent mitoses	
1950	11.17	11	8,1	8,2	1,0	
	12.9	8	8,3		1,0	
1951	4.19	8	6,5	7,9	2,4	
	4.27	10	9,3		1,0	1,6
1952	4.9	7	3,3	13,1	2,0	
	5.19	7	8,6		2,6	
	7.15	13	26,5		0,4	
	7.21	7	14,0		1,8	

The percentage of abnormal nuclei agrees with our data prior to 1952. Even if a slight increase in the frequency of abnormal cells might be read out of KLEIN's data, certainly no general shift has occurred as it did in our material. The appearance of the tumour pictured by KLEIN (1951 a, Fig. 43, p. 558) agrees with our strain before 1952. It is strikingly different in appearance from our strain after June 1952 (Fig. 2 *c*, *d* of the present paper).

The data go to show that a pronounced shift has occurred between 1951 and 1952 in the property of normal formation of resting nuclei in the Philadelphia subline of 6C3HED. Simultaneous shifts in other qualities of the tumour, such as increase in the yield of ascitic fluid and a possible slight upward shift in mitotic index, are also evident from observations during routine transfer.

The following account will be centered on an analysis of the cytological mechanism by which the nuclear fragmentation comes about, with emphasis on the question whether the abnormal multinucleate cells

represent degenerative stages of the tumour; this would imply that in certain cases (as Table 1, 1952 sample 7) very few cells would belong to the »germ line» of the tumour. The alternative is that multinucleate cells and cells with lobated nuclei are able to continue their mitotic activity. According to this second alternative, they must be able to collect the chromosomes from the various micronuclei or lobes of the same cell on to one normal bipolar spindle, whereupon the course of mitosis must continue normally. In this case, the only abnormal feature would be the process of formation of resting nuclei, the rest of the mitotic process including the centrosomic behaviour, proceeding without error. The present analysis will decide in favour of the latter alternative.

THE MITOTIC CYCLE OF THE ABNORMAL CELLS.

Resting stage. — The appearance of living 6C3HED cells, photographed in phase contrast, is seen in Fig. 2 *a* and *b*. In the former picture (from December 15, 1951) all the malignant lymphocytes clustered around a normal macrophage contain one smoothly rounded nucleus, while each cell in the latter picture (from August 26, 1952) contains a cluster of nuclear lobes and/or micronuclei. Fig. 2 *c* and *d* shows two samples of tumour cells fixed in aceto-orcein after the shift in nuclear type. In making smear preparations of this kind considerable pressure has to be exerted on the coverslip. This results in flattening of micronuclei and nuclear lobes after their displacement from their original clustered arrangement. Acetic fixation and the smear technique brought about a seeming increase in cell size (Fig. 2 *c*, *d*); in reality the whole of Fig. 2 is reproduced at the same magnification. In Fig. 2 *c* one diploid and in Fig. 2 *d* one approximately tetraploid metaphase are seen. An anaphase, one cell with pycnotic nuclei, and 2 non-malignant leukocytes are also visible in addition to the numerous resting nuclei of malignant lymphocytes. It is seen that all transitions occur between normal resting nuclei, such which have several nuclear lobes, and such in which the nuclear content is divided among many micronuclei. In Fig. 3 *a*—*k* instances of such nuclei are drawn. The morphological structure of the nuclear content seems normal in these cases. Often the resting nuclei contain a number of heteropycnotic bodies; heterochromatin is, however, not especially well-defined in this tumour.

In addition to this cell type a varying frequency of cells with a clearly pathological appearance is present. Such cells are the pycnotic cells of Table 1, a few instances of which are pictured in Fig. 3 *l*—*o*. They usually contain many small strongly stained nuclei. In some cases

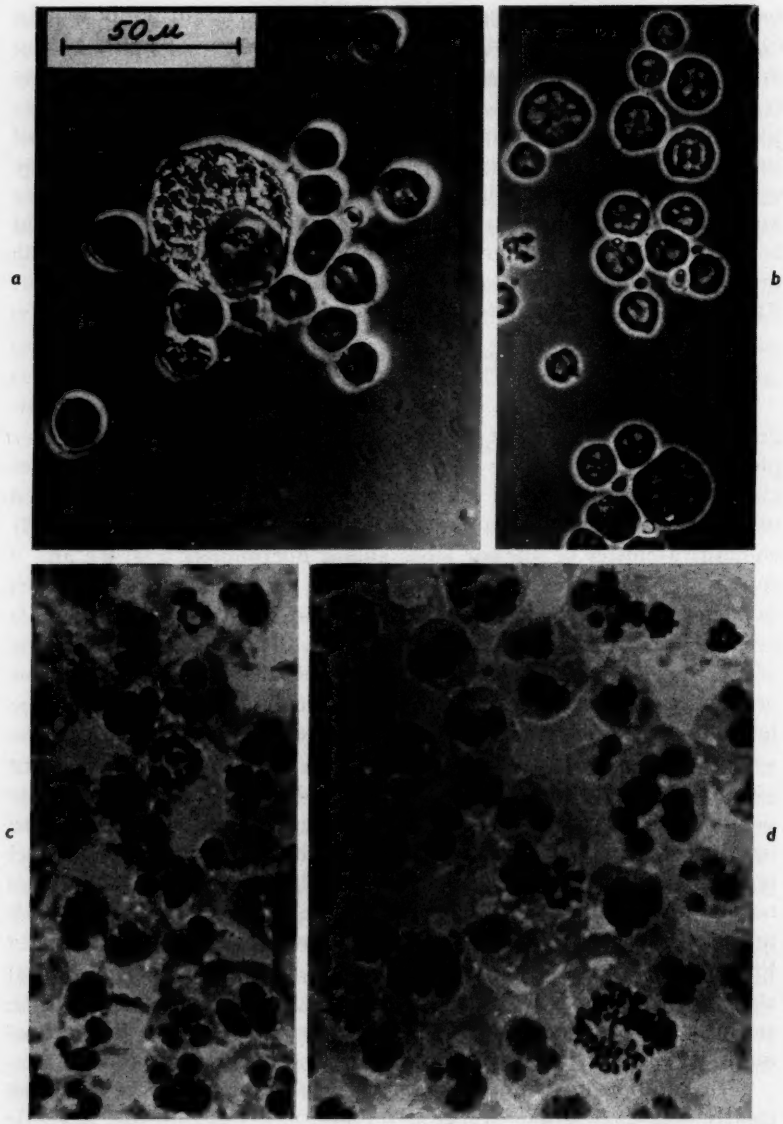

Fig. 2. General appearance of 6C3HED cells: *a*, before; *b*, *c*, *d*, after the shift in nuclear behaviour; *a*, *b*, living cells; *c*, *d*, cells fixed in aceto-orcein, in both cases photomicrographs in phase. — × 540.

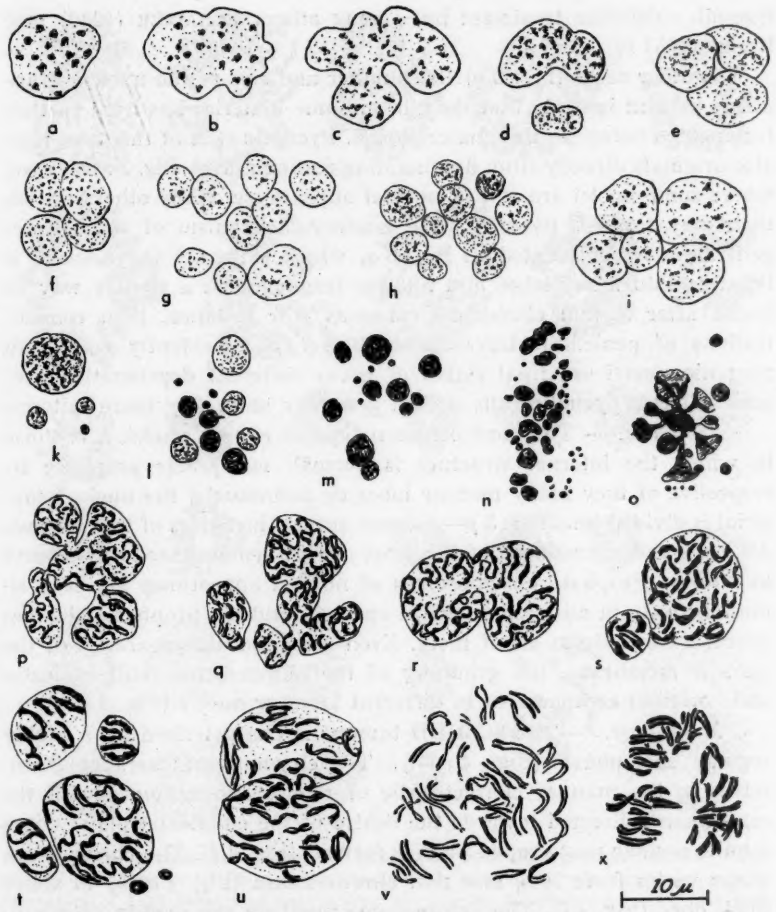

Fig. 3. *a*—*o*: resting nuclei, *p*—*x*: prophases. *a*: normal resting nucleus; *b*—*c*: lobated nuclei; *d*—*k*: resting stages with micronuclei; *l*—*o*: more or less pycnotic nuclei; *p*—*u*: prophases with lobes and micronuclei; *v*—*x*: transition to metaphase. — × 1300.

it was shown that many of these nuclei correspond to one metaphase chromosome, the daughter halves of which have not separated. They constitute the continuation of such mitoses as Fig. 4 *x*. This abnormality is more frequent in certain other tumours (as, for instance, in the DBA lymphoma). Its mechanism of origin is of a c-mitotic nature, and similar cell types have been induced on a big scale in ascites cells

through colchicine treatment by, among others, SCHAIRER (1940) and KLEIN (1951 b).

In other cases (Fig. 3 *n*) the number and size of the nuclear fragments present indicate that the chromosome material has been further fragmented before forming micronuclei. Pycnotic cells of this type may also originate directly from degenerating resting nuclei. Fig. 3 *l* is a case, where some nuclei are still of normal appearance, while others are on their way towards pycnosis. Still another mechanism of nuclear degeneration is represented in Fig. 3 *o*, where evidently the nucleus is bursting into many lobes and nuclear fragments in a similar way as found after certain chemical treatments (for instance, high concentrations of penicillin; LEVAN and TJIO, *l. c.*). Evidently cells with pycnotic nuclei are final stages of many different degenerative processes. These pycnotic cells do not generally enter any more mitoses.

Prophase. — The most common type of resting nuclei, *i. e.* those in which the internal structure is normal, may enter prophase irrespective of how many nuclear lobes or micronuclei the nuclear material is divided into. Fig. 3 *p*—*x* shows several instances of this process. All lobes and micronuclei of the same cell are synchronized with respect to mitosis. Prophase chromosomes of normal appearance are seen simultaneously in all micronuclei in one cell, and the prophasic changes proceed normally in all of them. Even after the disappearance of the nuclear membrane, the grouping of the chromosomes still indicates their original arrangement in different lobes or nuclei (Fig. 3 *v*, *x*).

Metaphase. — The 6C3HED tumour is characterized by a highly regular metaphase (Fig. 4 *a*—*i*). The chromosomes arrange themselves in the manner characteristic of mouse chromosomes with the centromeres directed towards the centre of the equatorial plate. Often a quite regular ring-shaped plate is formed (Fig. 4 *f*). The chromosome shape varies from long and thin chromosomes (Fig. 4 *a*, *b*) to short, thick ones (Fig. 4 *i*). The chromosome numbers are readily determinable; they gather around 40, 6C3HED being a diploid tumour (HAUSCHKA and LEVAN, 1951). The detailed distribution of chromosome numbers will be discussed in another paper in relation to the conditions of other tumours. Although the chromosome morphology and chromosome numbers of the metaphase plates pictured in Fig. 3 *a*—*i* are rather accurate, the pictures are intended more for showing the general appearance of this mitotic stage in 6C3HED than for detailed chromosome analysis.

All of the several hundred metaphase plates inspected during this

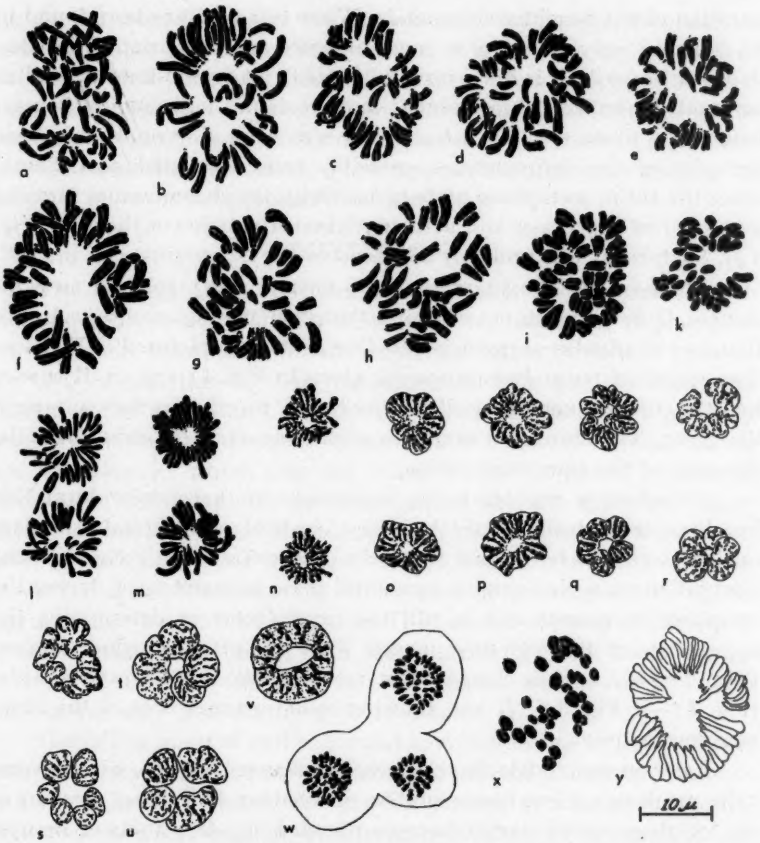

Fig. 4. *a—i*: normal metaphase; *k*: metaphase with sticky chromosomes; *l—m*: normal anaphase; *n—p*: normal telophase: *q—v*: telophases with varying degree of formation of karyolobes and karyomeres; *w*: tripolar telophase; *x*: metaphase with strongly contracted, »balled» chromosomes; *y*: metaphase plate with high degree of stickiness. — × 1100.

study were regular and bipolar. Somewhat deviating metaphases were met with occasionally, however. One type (Fig. 4 *x*) has already been mentioned in connection with the origin of pycnotic resting stages. It is a type of metaphase chromosomes often met with in tumour material. Its short, contracted, almost spherical chromosomes are very characteristic. As mentioned, it constitutes a degenerative type often leading over to pycnotic multinucleate cells without any anaphase se-

paration of the daughter chromatids. While this type has been found to be comparatively common in some tumours (as, for instance, in Ehrlich and Krebs 2), it is quite rare in 6C3HED, As is well-known, similar contracted, »balled» chromosomes may be found occasionally in normal mouse tissue too. Fig. 4 *k* shows a somewhat abnormal metaphase grouping of the chromosomes, probably caused by stickiness. Sometimes the entire metaphase plate turns sticky, the chromosomes forming a number of aggregates still in their original orientation on the plate (Fig. 4 *y*). Such cases will probably also lead to the degeneration of the cell.

Anaphase. — The anaphase too is normal and bipolar in most instances. Only some ten out of over a thousand anaphases observed were found to be tripolar or quadripolar. One of them is pictured in Fig. 4 *w*. Two stages of normal anaphase are given in Fig. 4 *l* and *m*. It is seen that the chromosomes are pulled more tightly together as they approach the poles. Therefore the anaphase plates are only of about half the diameter of the equatorial plates.

Telophase is regular in its beginning. No karyomere formation has been seen during early telophase, instead one normal telophase nucleus seems to be formed regularly. The characteristic chromosome orientation on a ring-shaped equatorial plate is maintained during the anaphase movement and is still the main factor in determining the appearance of the telophase nuclei. They are often doughnut-shaped (Fig. 4 *n*—*s*), a basic shape often recognizable in the resting nuclei (Fig. 4 *t*—*v*, Fig. 3 *e*—*f*) and showing radial arrangement of the chromosome material.

Later on during telophase significant changes happen, which eventually result in nuclear fragmentation or lobation. Individual portions of the telophase nuclei start to become rounded off, deep folds or fissures developing in between such portions (Fig. 4 *o*—*q*). Gradually entire portions separate from the rest of the nucleus forming free micronuclei. In extreme cases the entire telophase nucleus disintegrates into 5 to 10 or more such micronuclei. In other cases the main part of the telophase nucleus may hang together, one or more small micronuclei being free.

It is hard to say which factor is the cause of this peculiar behaviour of the ripe telophase nuclei. It seems that already the incidental grouping together of the chromosomes at anaphase has some influence on the later development. Such nuclear portions as are separated either as lobes or as micronuclei seem to contain chromosomes which through their anaphase orientation were predisposed to form groups of their own. The groups may be seen rather early during telophase but they

are cut off from the rest of the nucleus only during late telophase, in fact most lobes and micronuclei seem to originate during the nuclear swelling which takes place during transition from telophase to interphase. The liberation of micronuclei at this stage gives interesting aspects on processes such as the development of the nuclear membrane and the formation of nucleoli. Unfortunately the technique used by us does not allow us to approach these problems.

DISCUSSION.

The present study has demonstrated that the abnormal multinucleate cells of the 6C3HED tumour do not represent degenerative cell types but constitute normally occurring stages in the life cycle of the cells of this tumour. The multinucleate cells and the cells with lobated nuclei are able to re-enter mitosis and go through a perfectly normal mitosis. The deviation from normal behaviour occurs during late telophase, at which time the origin of several nuclei or nuclear lobes out of a normal telophase nucleus first becomes apparent. Naturally we do not know whether this phenomenon implies a *de novo* formation of nuclear lobes or fragments from a common telophase nucleus, or whether it just constitutes the falling apart of karyomeres held tightly together since the start of telophase. The general swelling of the nuclei at early resting stage might be a factor likely to bring about the separation of such karyomeres. It must be pointed out, however, that no karyomeres were seen during early telophase.

The falling apart of earlier formed karyomeres has certain parallels with the karyomere formation known from early cleavage mitoses in many animals, in which this process goes on as a perfectly normal phenomenon. The formation of karyomeres, however, is generally apparent from the very beginning of telophase. It is often ascribed to the rapid sequence of mitoses in the young embryos. In our case the factor of rapid sequence of mitoses may be of only minor importance, the mitotic frequency of samples with normal cells being only slightly less than the values for the population with multinucleate resting cells (mean of 1,42 *versus* 1,28).

The shift in frequency in the occurrence of this abnormality found between 1951 and 1952 in the Philadelphia strain of 6C3HED is possibly a selective phenomenon. One could speculate whether the condition of the nuclei to be separated into karyomeres or karyolobes might constitute a favourable condition under certain circumstances, and consequently be of positive survival value. For example, the slight in-

crease in mitotic index may be influenced by the obviously changed relationship of total nuclear surface to volume arising through fragmentation of the nucleus, with consequent effects on metabolic activity. In this connection it is of considerable interest that the important coenzyme diphosphopyridine nucleotide is produced almost exclusively in the nuclear fraction of normal C3H mouse cells recently studied by HOGEBOOM and SCHNEIDER (1952). Through some such selective mechanism the multinucleate cells would be favoured giving rise to a new population mode.

Acknowledgement. — This investigation was supported in part by a grant from the National Cancer Institute, U. S. Public Health Service.

SUMMARY.

The mitotic cycle in a strain of 6C3HED lymphosarcoma of the mouse, in which a shift has occurred towards a high frequency of resting cells with micronuclei or lobated nuclei, is described. It is shown that cells with this abnormality regularly undergo normal mitosis. The abnormal condition of their resting nuclei originates at the nuclear swelling during late telophase, when the nuclei have a tendency to fall apart into several micronuclei or form nuclear lobes. This seems to be the only error of the mitotic process, all other stages being largely regular. The formation of micronuclei in this case has thus nothing to do with any action of multipolar spindles, nor with the ordinary karyomere formation at early telophase.

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X-RAY MUTATIONS IN APPLES AND PEARS

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INTRODUCTION.

IN 1944 some experiments with X-ray treatment of fruit tree scions were started at the Balsgård Fruit Breeding Institute by Å. GUSTAFSSON and FR. NILSSON. Some preliminary results from 1944—48 have been published by GRANHALL, GUSTAFSSON, NILSSON and OLDÉN (1949), and additional data have later been given by the present author (GRANHALL, 1949, 1951). Since 1949 other mutagenic agents, *i.e.* neutrons and radioactive isotopes (*cf.* also EHRENBERG and GRANHALL, 1952), have also been included in the mutation experiments at Balsgård. The present paper, however, will be confined to a description of the cases of X-ray induced mutations hitherto discovered in the apple and pear material.

The author wishes to express his gratitude to the Knut and Alice Wallenberg Foundation for grants made, to Professor ÅKE GUSTAFSSON for directions and agreeable co-operation, and to the members of the Balsgård staff, especially Mr E. J. OLDÉN and Mr K. A. ANJOU, for their assistance in the experiments. The performance of the irradiations has been entrusted to Dr. A. FORSSBERG at the Radiophysics Institute, Stockholm, during the years 1944—51. From 1952 onwards Professor A. MÜNTZING has placed the new X-ray equipment of his institute at our disposal.

PRIMARY RADIATION EFFECTS.

In a series of papers, mentioned above, the immediately visible effects of the X-ray treatment have already been described. They can be summed up as follows. Scions of diploid apples and pears, treated with 5000 r-units of X-rays and then grafted, showed a surviving per cent of about 50 as compared with an average of about 80 in untreated control scions. Triploid and, especially, tetraploid varieties showed a higher degree of viability after the same treatment.

The growth of the surviving X-ray scions was markedly delayed. Irregular leaves were very often formed from the developing buds. Later

in the season bifurcated leaves and shoots also appeared in several cases. As seen from Table 8 in the paper by EHRENBERG and GRANHALL (1952), 60 per cent of the diploid, 20 per cent of the triploid and 4 per cent of the tetraploid apple scions, surviving after 5000—7500 r, gave rise to bifurcated shoots. In pears a frequency of 16 per cent bifurcated shoots appeared as an average among diploid and triploid scions receiving 5000 r (Table 10, *l. c.*). Sometimes these »primary effects», originating from damage to the vegetation points, were repeated during the second, or even the third, season after treatment and grafting. At other times bifurcated shoots appeared quite suddenly after several years' normal growth, in the extreme so late as the sixth season (*cf. l. c.*, p. 414).

MUTATIONS HITHERTO OBSERVED.

Special interest has of course been devoted to the detecting of possible bud sports, caused by the irradiation. The first supposed cases were noted in 1948 (GRANHALL *et al.*, 1949). Three of them (red fruit colour in Cox's Orange apple, pointed shape and late fruit maturity in Williams pear) have been the object of some further observations at Balsgård, but unsatisfactory fruit settings have still prevented definite conclusions.

On the other hand, a series of undisputable vegetative mutations have been obtained during later years in apples and pears after X-ray treatment. In the following a description of some typical examples will be given.

(1) Among seven scions of the diploid Esperens Herre pear (Belle Lucrative) which had been treated with 5000 r in 1947 and grafted on an older pear tree (»Tree K» = »Sugar Pear» or Kleine Gelbe Frühbirne; *cf.* GRANHALL *et al.*, 1949, p. 273), one of them gave two normal and two striped fruits in 1949 (Fig. 1; *cf.* GRANHALL, 1949). The fruits were, however, blown off their spurs before mapping of the branch could be carried out. Next summer, 1950, there were twelve pears on the scion (named VII) and it was now quite clear that the mutation had the nature of a sectorial chimaera. As seen from Fig. 3, two buds developed from the original scion. One of them (branch VII:*a*) carried five fruits, all normal. The other shoot was divided into four branches (named VII: *b*—*e*), one of which (*b*) had four normal pears; two (*c* and *d*) had three striped pears (two and one, respectively); the remaining one (*e*) had no fruits. In 1951 there was a rich bloom and

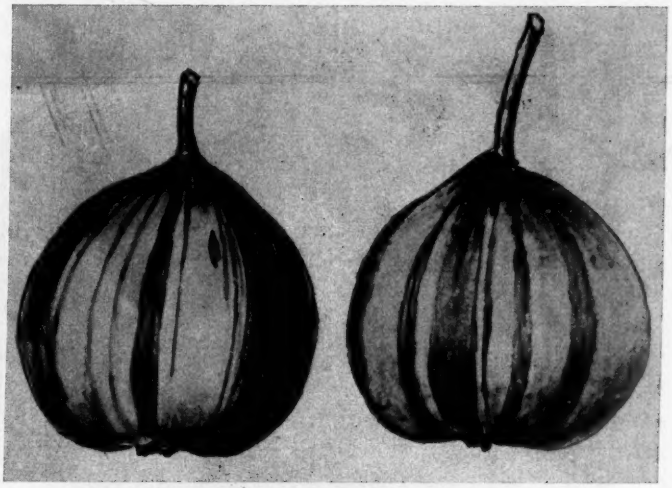

Fig. 1. Striped fruits from branches *c*—*d* of Esperens Herre scion No. VII, harvested in 1949. (Drawing by E. J. OLDÉN.)

formation of fruitlets in the different branches of the scion, but owing to an accident at the spraying of the orchard in July the branches *b*—*e* were broken loose. All suitable buds, 101 in number, from the branches were immediately transplanted to 14 nursery trees with careful registration of their original sites. In 1952 about half of these buds are growing, and the experiment will thus serve to elucidate the possibilities of cultivation and propagation of mutations from chimaeral shoots. This procedure will of course be more complicated, if the mutations turn out to be periclinal chimaeras as well, in analogy with the tetraploid »giant sports» of apples, described by DERMEN (1951) and others.

Spontaneous bud sports with striped fruits have been reported in pears in a great number of cases (SHAMEL and POMEROY). Soon after the striped X-ray mutation of Esperens Herre was found at Balsgård, Mr IVAR NILSSON, gardener, informed me that a 50-year old pear tree of the variety Williams (=Bartlett) at Helmershus near Kristianstad had fruits with the same type of green and yellow striation (Fig. 2). A closer investigation of this tree has revealed its chimaeral character, inasmuch as mutated and normal fruits appeared intermingled with each other, sometimes even in the same cluster. The sport also seemed to be connected with certain deficiencies in the chlorophyll content of other parts of the tree. Many shoots have thus a lighter green leaf co-

Fig. 2. Cluster of striped fruits from the spontaneous mutation of Williams pear at Helmershus.

lour and, in addition, a reddish tint of shoot tips and leaf stalks. The striping of the fruits comes in sight already at the end of June in the early fruitlet stage of this Williams sport in contrast to the behaviour of the X-ray mutation in Esperens Herre, the stripes of which are not quite obvious before fruit ripening in October. There also seems to be very little difference, if any, between sport and normal leaf and shoot colour in this latter case.

(2) Another scion of Esperens Herre pear, belonging to the same treatment (1947; 5000 r) and grafted on the same tree (»Tree K») gave several normal and two quite russeted fruits in 1950. The scion (named III) had only normal fruits in the previous years 1948 and 1949. In 1951 the scion was mapped (Fig. 4). There were two developing buds from the originally grafted scion. The base shoot (branch III: *a*) had three normal fruits. The top shoot was divided into seven longer or shorter twigs (branches III: *b*—*h*), four of which had normal fruits (*c*, *e*, *g*, and *h* with 7, 3, 2, and 1 fruits, re-

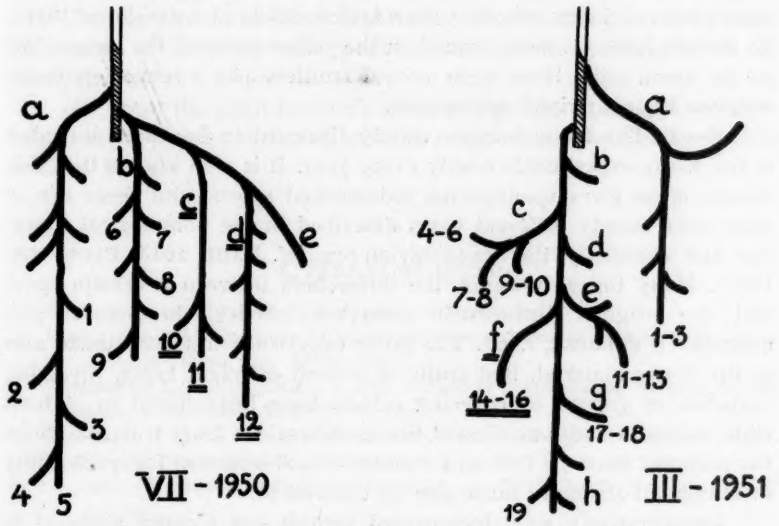

Fig. 3. Skeleton drawing of Esperens Herre scion No. VII in 1950. Branches *c* and *d* carried the striped fruits (Nos. 10—12). — Fig. 4. Skeleton drawing of Esperens Herre scion No. III in 1951. The russeted fruits (Nos. 14—16) belonged to branch *f*.

spectively). In branch *f*, however, there were three fruits, brown russeted all over and recalling »Gray Pears» (variety Grise Bonne) but with the typical shape of Esperens Herre. In analogy with the striped Esperens Herre mutation the russeted one is consequently a sectorial chimaera.

Spontaneous russeted pear sports are listed by SHAMEL and POMEROY, and also by other observers.

(3) The acquaintance with the spontaneous chlorophyll deficient Helmershus Williams, described above, initiated the idea that a further break-down of the chlorophyll gene system could be obtained by X-raying of this sport. In the 1950 irradiation experiments 14 scions of the Williams sport were consequently treated with 5000 r. In the first season the result was limited to some primary leaf and shoot defects of the ordinary X-ray type, but in 1951 four of the scions showed chimaeral structure with white-yellow leaves in single clusters or whole young shoots. Some leaves were half yellow, half green. In the autumn the yellow leaves finished their growth later, but the shoots nevertheless stood the winter well. The yellow leaves reappeared in 1952 from the

same places and new, wholly yellow-leafed shoots also developed there. No flowers have yet been formed on the yellow parts of the shoots, but on the green parts there were several fruitlets and a few ripen fruits with the typical striped appearance.

(4—6) The triploid apple variety Gravenstein has been included in the X-ray experiments nearly every year. It is well known that this variety often gives spontaneous red-cheeked sports, and there are at least some twenty different cases described in the pomological literature and, especially, the Scandinavian one (*cf.* DAHL, 1943; PEDERSEN, 1937). Many times, however, the differences between a certain sport and the original Gravenstein seem very difficult to discover and maintain (PEDERSEN, *l. c.*). The latter experience has been made also in the X-ray material. Red fruits of several different types, involving variation of ground or covering colour, have been found in at least eight cases. In order to sift out the modifications from true mutations the material must be followed during several seasons. The probability of chimaeral structure must also be considered.

Comparatively well documented records are already attached to the scions 0612 A, 0614 H, and 0614 L. These scions were all treated with 5000 r in 1948 and top-worked on two trees (0612 and 0614) of the tetraploid 37/54 with E. M. IX as root-stock. In the same trees there were, in addition, 21 other Gravenstein scions with the same treatment, all evidently or probably normal, with the possible exception of three doubtful ones (0612 G, 0612 K, 0614 I), which will be studied further. There are at least three different types of red apples represented in the X-ray material of Gravenstein, namely, crimson flush with broken stripes of the same or slightly darker tint, crimson flush and very dark blood-red stripes, and, finally, a lighter and very fiery cherry flush with but few stripes. It seems necessary to devote to these colour variations and their genetical stability closer investigation in comparison with the sports of Gravenstein already described.

SUMMARY AND CONCLUSIONS.

(1) A series of X-ray induced vegetative mutations have been produced at Balsgård in pears and apples.

(2) The present paper gives a description of six of these mutants, namely, green-yellow striped and brown russeted fruits of Esperens Herre pear, foliage chlorophyll defects in a form of Williams pear, and three cases of red Gravenstein apple.

(3) The vegetative X-ray mutations have the structure of sectorial chimaeras. If they are also periclinal chimaeras remains to be investigated.

(4) From the plant breeder's point of view the results hitherto obtained seem very encouraging and the experiments are continued on a broader basis.

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SOME COLCHICINE EXPERIMENTS WITH HOPS (HUMULUS)

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THE material used for the experiments was commercial samples of *Humulus japonicus* and seeds harvested in 1938 from a type of *Humulus lupulus* designated 1014, produced at this laboratory. Pollination of the latter seeds by one of the male plants grown in the experiment gardens, was uncontrolled.

For each experiment 100 seeds were used. The seeds were soaked in water for 24 hours and then placed in a solution of colchicine at different periods before the seeds were put to germinate. The resulting plants were isolated in pots, and root-tips were fixed from all those plants which had the slightest abnormal appearance. The fixations were made in Lewitsky's solution and the slides were stained in gentian violet. The results are shown in Tables 1 and 2, where the tetraploid chromosome numbers indicate tetraploid roots or sectors.

TABLE 1. *Humulus japonicus.*

Percentage of colchicine	Number of 24 hours soaked	Number of plants examined	Somatic chromosome numbers
0,25	1	1	16
		2	17
0,5	1	2	16
		1	17
		2	17 + 34
0,25	3	8	16
		3	17
		1	17 + 34
0,5	3	4	16
		1	16 + 32
		1	17 + 34

Altogether 15 plants had 16 chromosomes, 1 plant 16 + 32, 6 plants 17, and 4 plants 17 + 34. The number of plants are too small to justify any decisive conclusion being drawn but the result may indicate that the male plants are more sensitive to colchicine than female plants.

TABLE 2. *Humulus lupulus.*

Percentage of colchicine	Number of 24 hours soaked	Number of plants examined	Somatic chromosome numbers
0,25	1	1	20
0,5	1	1	20
0,25	2	2	20
0,5	2	1	20
0,25	4	4	20
		1	30
0,5	4	1	20
		1	40

The seeds treated for 8 times 24 hours did not germinate.

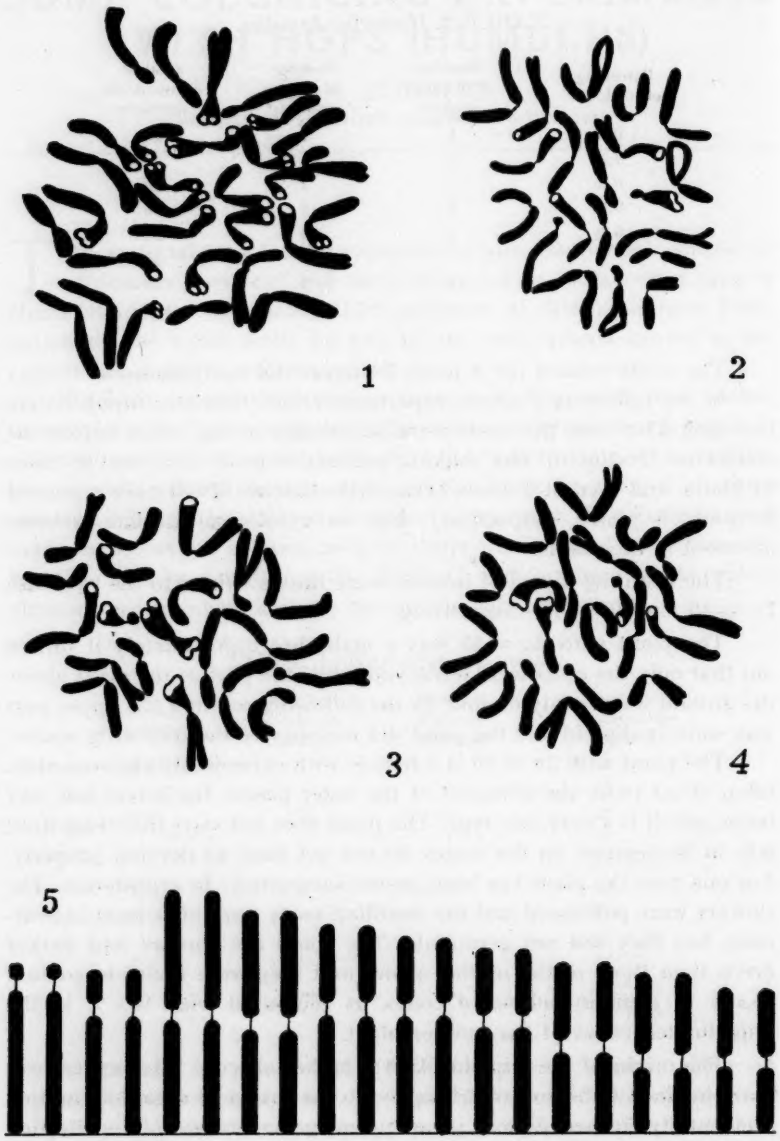
In the following year the experiments with *Humulus lupulus* were repeated. This time the seeds were not soaked in tap water before the colchicine treatment. The soaking periods were 3, 4, 5, and 6 times 24 hours, and even at 6 times 24 hours the batches of 100 seeds produced 69 and 73 plants, respectively, but no cytological deviations were observed in the plants.

The resulting plants of interest were thus confined to the two with $2n = 40$ and $2n = 30$, respectively.

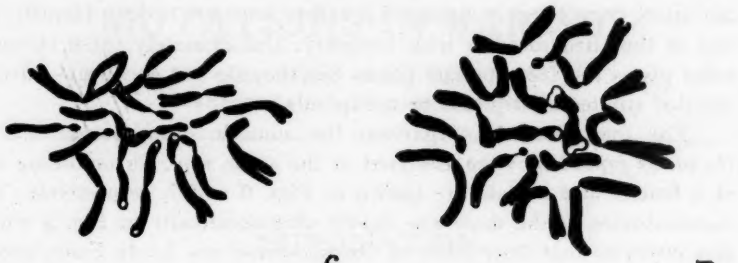
The plant with $2n = 40$ was a male, but unfortunately it turned out that only the roots were tetraploid while the part of the plant above the ground was mainly diploid. In the following summer the upper part was entirely diploid and the plant did not survive the following winter.

The plant with $2n = 30$ is a female with exceedingly vigorous stem often about twice the diameter of the sister plants, the leaves are very large, and it is a very late type. The plant does not start flowering until late in September, so the cones do not get time to develop properly. For one year the plant has been grown successfully in greenhouse. The flowers were pollinated and the resulting seeds were of normal appearance, but they did not germinate. The cones are smaller and darker green than those of the mother plant, and they were found to contain 16,0 % of resin in pollinated cones, as compared with 16,8 % in the *un*pollinated cones of the mother plant.

The origin of the triploid plant is rather obscure. The writer feels more inclined to believe that it is due to the fusion of a haploid and an accidentally formed diploid gamete during the process of fertilization and that its observation after the colchicine treatment is purely accidental. The genetic constitution would thus be either XXX or XXY.



Figs. 1—5. Somatic chromosomes of *Humulus lupulus*. — Fig. 1, triploid. — Figs. 2—4, diploid. × ab. 3500. — Fig. 5, schematic chromosome idiogram of the diploid. × ab. 7000.



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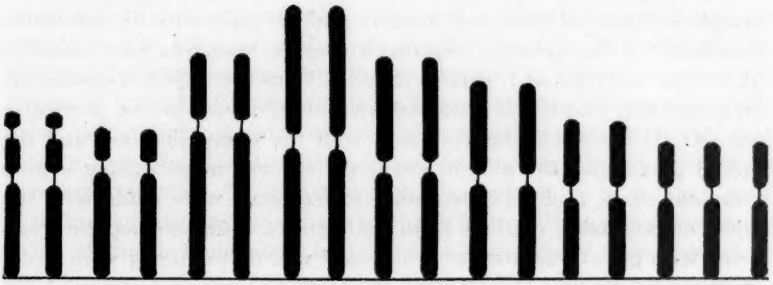

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Figs. 6—8. Somatic chromosomes of *Humulus japonicus*. — Fig. 6, female. — Fig. 7, male. × ab. 3500. — Fig. 8, schematic chromosome idiogram of the male. × ab. 7000.

The fixed material was insufficient for detailed cytological studies. In an attempt to solve this question by examination of further material detailed studies were made of some of the best looking chromosome plates from the diploids.

The somatic chromosomes of *Humulus lupulus* have been studied by WINGE (1929) and »the twenty chromosomes in a good female somatic nuclear plate» were measured without it being possible to demonstrate somatically the sex chromosomes.

In Figs. 2—4 the individual chromosomes were drawn separately and measured and it was then possible to arrange the chromosomes in groups according to their morphology. Two pairs are easily separated out, due to the very unequal arms, and are seen to the left in Fig. 5,

but apart from these it appeared a rather hopeless task to identify the rest of the chromosomes with certainty. Unfortunately these chromosome plates are from female plants but they do not encourage further detailed studies of triploids or tetraploids.

For the sake of comparison the somatic chromosome sets of *Humulus japonicus* were analysed in the same way. Chromosome sets of a female and a male are shown in Figs. 6 and 7, respectively. The chromosomes of the male are shown diagramatically in Fig. 8 which demonstrates that four pairs of chromosomes are fairly easily recognizible and that the chromosome repeated three times is to be found between the smallest of the chromosomes.

The experiments recorded have shown that it is possible to obtain tetraploid tissue in *Humulus lupulus* and *H. japonicus* by colchicine treatment. In the present experiments only chimaeras were obtained apart from the triploid *Humulus lupulus*. With the object of producing tetraploid hop plants the following two observations are of particular interest: (1) the particular chimaera with the tetraploid roots and the diploid part above the ground did never succeed in producing a pure tetraploid clone, and (2) some other experiments were made with the object of producing cuttings from the stem of a diploid hop by treatments with growth-substances. The result was that cuttings were easily produced in this way but the resulting plants were not perennial but died during the winter time. These two observations taken in conjunction indicate that successful polyploids of hops must be produced from a chromosome doubling very early in the process of germination. If it is justifiable to draw any conclusions from the comparison between the diploid and triploid plants described, tetraploid plants will probably be much too late to be of any commercial interest under the present climatic conditions.

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HETEROZYGOSITY IN ERECTOIDES MUTATIONS IN BARLEY

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I. INTRODUCTION.

IN order to gain a deeper understanding of the heterosis problem it is necessary to learn to know, as far as possible, the nature of the genes and of their actions and interactions; a knowledge which may be said to be the ultimate goal of all genetic research. The artificial induction of mutations has offered new possibilities for the study of the effects of individual genes and of their interactions with the rest of the genotype. In mutants obtained from pure lines it is possible to determine exactly how the mutated gene reacts, in homozygous as well as in heterozygous state, in a completely constant or in a varying gene environment. Among other work, the effects of some homozygously lethal chlorophyll deficient mutations in barley have been studied in their homozygous and heterozygous states and in varying environments (GUSTAFSSON *et al.*, in a series of papers 1946—1951). It was possible to demonstrate that in some cases the heterozygote is superior to the normal homozygote. It must be of interest to make similar investigations in regard to the whole series of fully vital, morphologically distinct induced mutations.

In barley — the plant most extensively studied with regard to the utilization of induced mutations in plant breeding — a large number of vital mutants of different types have been isolated. Especially common are mutations in ear-density, the so-called *erectoides* mutants (*cf.* HAGBERG, NYBOM and GUSTAFSSON, 1952). So far such mutations have been observed in about 60 cases; in all of them the internodes of the ear are shorter in the mutant than in the parent and in most cases this character is correlated with an increased stiffness of the straw. These mutations have been, and still are, the subject of an analysis in regard to their location: whether they are multiple alleles or they belong to different loci, linked or non-linked (NILSSON-EHLE, 1939; HAGBERG, NYBOM and GUSTAFSSON, *l. c.*); and also in regard to their cytology (HAGBERG and TJIO, 1950, *etc.*). An important part of the analysis of the erectoides

mutant genes is a study of their effects in homozygous and heterozygous state and of their interactions in different combinations.

When planning the study on heterosis, begun by the author in 1945, the question of monofactorial heterosis was considered to be one of the main problems (HAGBERG, 1952 a and 1953), and it was quite natural that the erectoides mutants available at Svalöf were chosen as material for this special phase of the investigation.

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II. MATERIAL AND METHODS.

Three different varieties of barley, all two-rowded and of *nutans* type, have been used in the mutation experiments at Svalöf (GUSTAFSSON, 1947). At first Gull (Golden barley) was treated with X-rays. Gull is a pure line from a land variety from the isle of Gotland. From this variety erectoides 1—11 were isolated. Erectoides 12—16 and 29 were obtained from Maja barley, a line from a cross Gull × Binder, which latter variety is a pure line from a Moravian land variety. Erectoides mutants 17—32, with the exception of 29, derive from Bonus barley, those with higher numbers either from this variety or from sib lines from the same cross: (Hannchen × Gull) × (Gull × Binder), Hannchen having the same origin as Binder.

In 1945 the author obtained F_2 material of the crosses *ert* 2 × Gull, *ert* 6 × Gull and *ert* 7 × Gull (for the symbol *ert*, see HAGBERG and TJIO, 1952). The F_2 seeds were sown in the spring of 1946, the object being a comparison between the three genotypes. In the following the symbols *AA*, *Aa* and *aa* will be used to denote normal homozygote, heterozygote and mutant homozygote, respectively, for any cross between parent and mutant. Several notations, measurements and weighings were made on the ripe plants, and in 1947 30 seeds from each lax eared plant were sown in order to determine the genotype of the F_2 plant and to repeat, in segregating progenies, the comparison between *AA*, *Aa* and *aa*. The seeds were individually spaced by marking boards, 30 seeds being sown 5 cm apart in each row with 15 cm between the rows.

In 1946 and 1947 crosses were made on a large scale between the two best — from a practical point of view — mutants from Gull, *viz. ert* 1 and *ert* 7, and the mother line. Crosses were also made within the

parent strains in order to obtain fully comparable material of parents and F_1. In 1949 *ert* 19 and *ert* 21 were crossed with one another and with their mother variety, Bonus, for a comparison between parents and F_1. The comparisons here mentioned have been made on material, sown by marking boards with 15 cm between the rows, 10 cm between the plants in the row and 15 plants to the row. The material has been handled in the manner described for similar comparisons in other barley material (HAGBERG, 1953). If the seed from a cross has not been sufficient for a full row the row has been completed with spring wheat, thus eliminating deficiencies in the stand as far as possible. Generally there have been far more than 15 seeds available of each combination and in such cases replicates have been used, submitting as far as possible all biotypes to the full variation of the soil (for details of the method, see HAGBERG, 1952 a). Each plant has been used as a variate in determining the *t* values used in estimating the statistical significance of differences observed.

As part of the greater program for an analysis of the allelism and linkage of the erectoides mutants diallel crosses have so far been made between all the erectoides mutants from Gull (G-ert), between the Bonus erectoides 17—28, 31—32 and also crosses between each G-ert locus and the B-erts mentioned. This work has been carried out during 1948—52 and the material from these crosses has been used for comparisons similar to those described above. The number of F_1 seeds in the large number of diallel crosses has only been 15—20 in each combination and no crosses within the parent lines have been made; the comparisons obtained from this material are, therefore, less reliable than those from the more complete material from the crosses specially planned for the present investigation.

III. THE HOMOZYGOUS ERECTOIDES MUTATIONS.

NILSSON-EHLE (1939, 1948), GUSTAFSSON (1941, 1947) and others have described the erectoides mutants, the common character of which mutants is the more or less dense ear. In the present study the density has been determined as the length of 10 internodes, from the base of the fifth kernel to that of the fifteenth. (BATEMAN and MATHER, 1951, used the length of 14 internodes and WEXELSEN, 1934, the average internode length of the whole ear.)

As seen from Table 1, different erectoides mutants have a very different internode length. There is a series of values from the com-

TABLE 1. *Internode length of erectoides mutants. 1951.*

Locus a		*Locus b*		*Loci only represented by one single allele*			
Ert a 6	19,8	Ert b 2	26,5			Ert h 25	28,8
» a 11	19,3	» b 4	26,2			» i 27	24,0
» a 19	20,3	» b 5	25,0	Ert 9 ?	23,3	» j 31	23,6
» a 21	23,1	*Locus c*		» e 17	15,3	» k 32	25,1
» a 23	21,3	Ert c 1	26,0	» f 18	26,4	*"Mother strains"*	
» a 28	25,6	*Locus d*		» 20	31,9	Gull	31,4
» a 29	19,8	Ert d 7	23,9	» g 24	22,7	Bonus	32,0

paratively lax ear of *ert* 2, which is, however, easily distinguished from the mother line, to the extremely dense ears, similar to the old so-called *zeocriton* type, which are represented in the table by *ert* 17.

If two ert genes from different loci are combined in one plant a very dense ear is obtained (*cf.* NILSSON-EHLE, 1939, 1948). Table 2 gives the density values for *ert* 1, *ert* 7 and their combination (*aabb*) as well as for the mother line, Gull. The table also gives the values for the different biotypes in F_2, homozygous and heterozygous, which values will be discussed later on. Studies of other similar combinations, and also of the accumulation of 3 or 4 ert genes, are under way but no definite values are so far obtainable.

GUSTAFSSON (1947) has tested the practical value of the different ert mutants and given several data about other characters than ear density and D. VON WETTSTEIN (1952) has analysed the morphological and anatomical causes of the superior stiffness of straw, but it is superfluous here to enter upon these questions.

It is important to note that the segregation data given in Table 3 show a monohybrid, 3 : 1, segregation for each ert gene. There is a slight but significant deficiency of homozygous recessives, which is a normal occurrence.

TABLE 2. *Internode length of different genotypes in the F_2 generation of the cross ert c 1 × ert d 7, and of the parents and the »mother strain», Gull. 1950.*

			F_2:			
Gull	*AABB*	32,0	*AABB*	33,3	*aaBB*	24,2
Ert c 1	*aaBB*	24,6	*AaBB*	31,6	*AAbb*	
Ert d 7	*AAbb*	25,1	*AABb*		*aaBb*	23,1
1 + 7	*aabb*	16,2	*AaBb*	31,1	*Aabb*	
					aabb	16,2

In some cases two or more ert mutations have been shown to have occurred in the same locus (HAGBERG, NYBOM and GUSTAFSSON, *l. c.*). In those cases it seems expedient to give the locus a special designation, *e. g.*, *a*, *b*, *c*, *etc.* and to denote the mutations occurring in them with the letter of the locus and the original number of the mutant. When *ert* 6, *ert* 11, *ert* 13, *etc.* are found to be alleles they are from then on designated as *ert a* 6, *ert a* 11, *ert a* 13, *ert a* 19, *ert a* 21, *etc.* Similarly we have *ert b* 2, *ert b* 4 and *ert b* 5; *ert c* 1 and *ert c* 39; *ert d* 7, *d* 14, *d* 15, *d* 33. When any one mutation has been found to be definitely non-allelic with the loci already designated, it is allotted a definite letter.

TABLE 3. *Segregation in some crosses between erectoides and Gull.*

Comb.	Type of ear lax	Type of ear dense		Chi^2	DF.
Ert b 2 × Gull	10647	3301		$13{,}23^{***}$	1
» a 6 × »	7065	2226		$5{,}37^{*}$	1
» d 7 × »	797	238		$2{,}22^{0}$	1
			Sum	$20{,}82$	3
Sum	18509	5765		$20{,}24^{***}$	1
		Heterogeneity		$0{,}58^{0}$	2

In no case so far studied two mutations have been proved to be identical; if they have happened in the same locus they usually represent distinctly different multiple alleles; the exceptions so far are the mutations *ert b* 2, *b* 4 and *b* 5. Reference is made, in the first hand, to the table in HAGBERG, NYBOM and GUSTAFSSON (*l. c.*) giving the ear density for the different alleles in locus *ert a*.

Ert d 7 is (as well as *ert c* 1) absolutely linked to a translocation point (HAGBERG and TJIO, 1950; TJIO and HAGBERG, 1951). In F_4 of the cross *ert d* 7 × Gull, *e. g.*, 233 plants were heterozygous for *ert d* 7; all of them except 2 were partially sterile and thus heterozygous also for the translocation, which conclusion was confirmed by a study of the next generation. Even the 2 nearly fertile plants — with more than 90 % seed setting — were found to be heterozygous for the translocation. All homozygotes for *ert d* 7 were fertile in F_4 and also their progeny in the next generation.

Besides *ert d* 7, *ert d* 14 and *ert d* 15 belong to locus *d*; the two latter mutants are clearly different in regard to length of straw. According to NYBOM (unpubl.), *ert d* 14 is homozygous for a translocation, which is not identical with the one linked with *ert d* 7,

although they probably have one breaking point in common. *Ert d* 15, finally, has no translocation, which shows that the mutations in this locus are at least not solely due to a position effect.

IV. THE HETEROZYGOUS ERECTOIDES MUTATIONS. MONOHYBRIDS.

The effect of the mutations in the heterozygous state has been most extensively studied in regard to *ert b* 2, *ert a* 6 and *ert d* 7 in segregating generations and in regard to *ert c* 1, *ert d* 7, *ert a* 19 and *ert a* 21 in comparisons between parents and F_1.

The necessity to determine the genotype of the plants by a study of their progeny has offered a fairly extensive material for a control of the segregation, as already mentioned. The data in Table 3 show that the three ert mutants included segregate in a manner clearly indicating monohybridism but also with a clear deficit in the homozygous recessive. There is no striking difference between the mutations in this respect and the material from all three has been pooled in calculating the heterogeneity. As seen from Table 4, the variation between F_4 families in the ratio of recessives is greater than accounted for by random sampling. The distribution of P-values for the ratios in individual families is distinctly skew, depending upon an excessive number of families with a large deficit of recessives.

TABLE 4. *Heterogeneity in segregation between different families in the material presented in Table 3.*

Heterogeneity	Chi^2	DF	P
between F_4 families within F_3 and F_2 fam.	937,56***	754	0,001
» F_3 » » F_2 fam.	150,71	129	0,20—0,15
» F_2 » » the cross	2,54	8	0,98—0,95
» different crosses	0,58	2	0,8—0,7

The results of the measurements on the segregating material as well as on the F_1's of the crosses *ert c* 1 × Gull and *ert d* 7 × Gull are found in Table 5. In this table absolute values are given for the normal homozygote, *AA*, while only relative values, with $AA = 100$, are given for *Aa* and *aa*. The relations between the genotypes vary considerably from year to year. The tillering seems to be fairly high in most *Aa*. In length of straw there is no difference between *AA* and *Aa*. On the other hand, the ear of *Aa* is in most cases shorter than that of *AA*. This is due to the fact that *A* is not completely dominant in its effect upon ear

TABLE 5. *Data from measurements on heterozygous and homozygous plants in crosses between ert and Gull.*

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Year	Gen.	10 cm btw. pl. *Ert c* 1 × G			Gen.	5 cm between plants *Ert b* 2 × G			*Ert a* 6 × G			*Ert d* 7 × G			Gen.	10 cm btw. pl. *Ert d* 7 × G		
		AA	*Aa*	*aa*		*AA*	*Aa*	*aa*	*AA*	*Aa*	*aa*	*AA*	*Aa*	*aa*		*AA*	*Aa*	*aa*
						Number of ears per plant												
1946					F_2	5,4	101	81	5,4	98	91	5,4	116	112				
1947					F_3	4,3	103	84	4,6	94	83	5,3	110	103	P and F_1	7,9	114	104
1948	P and F_1	4,2	95	99	F_4	3,0	100	83	3,4	102	98	3,4	93	89	»	4,2	101	101
						Length of straw cm												
1946					F_2	101	100	94	101	101	97	96	105	105				
1947					F_3	62	101	91	61	98	86	61	101	91	»	67	99	96
1948	»	78	100	94	F_4	82	100	96	83	99	95	80	100	101	»	78	101	103
						Length of ear cm												
1946					F_2	7,0	99	77	7,0	95	63	7,0	105	93				
1947					F_3	7,3	97	81	7,3	95	66	7,1	98	76	»	8,7	97	75
1948	»	8,2	97	80	F_4	7,1	97	78	8,1	96	66	8,1	93	76	»	8,2	97	78
						Weight of plant g												
1946					F_2	10,1	99	72	7,3	125	110	11,1	121	128				
1947					F_3	7,7	101	80	7,8	92	77	9,1	113	90	»	18,6	115	92
1948	»	9,0	97	96	F_4	6,6	98	83	7,8	104	95	8,2	83	83	»	9,0	100	103
						Weight of grain per plant g												
1946					F_2	5,0	98	74	3,9	122	90	5,3	103	114				
1947					F_3	3,4	99	64	3,7	91	70	3,5	102	72	»	10,0	107	84
1948	»	5,3	89	93	F_4	3,3	98	80	3,8	104	90	3,9	74	84	»	5,3	89	95
						Seed setting %												
1947					F_3	91,0	98	76	92,2	100	87	86,9	82	76				
1948	»	96,5	86	101	F_4	96,6	100	100	96,1	101	98	95,8	79	101	»	96,5	79	100
						Number of florets per ear												
1947					F_3	19	103	104	20	101	103	20	103	91				
1948	»	24	100	100	F_4	23	100	98	23	100	100	24	99	97	»	24	107	96
						1000 grain weight g												
1947															»	45,4	107	100
1948	»	44,7	107	96											»	44,7	106	97

density, so that the internodes of the ear in *Aa* are somewhat shorter than those in *AA*. In total plant weight and yield of grain per plant the relations vary strongly from year to year, and there is no significant average difference in favour of *Aa*. The seed setting is normally good except in the *Aa* biotypes which are also heterozygous for a translocation. The summer of 1947 was extremely dry and in this year the seed setting was remarkably low, especially in *aa*. The seed setting of the mutants studied seems to be more sensitive to the environment than that of normals. The number of florets per ear is about the same for all genotypes in a given year. Possibly the *Aa* type for *ert d* 7 has a somewhat higher number of florets than the *AA* type. In one of the three sets of data, however, there is no difference, and there is probably no reason to put too much value on the difference.

The seed of the heterozygotes of *ert c* 1 and *ert d* 7 is significantly larger than that of the normals. Both these heterozygotes are partially sterile, however, because of their heterozygosity for a translocation, and the lower number of grains per ear may easily explain their greater weight.

On the whole, in the material so far reported there are no clear and unequivocal instances of monofactorial heterosis or superdominance.

NYBOM (1950) has described a case of superdominance in barley. He writes, however, that »the empirical basis, *i. e.* the number of plants analysed, is not very large and certain objections may be raised against the experimental conditions». For this reason the present author has repeated some of his crosses on a considerably larger scale and grown F_1 and parents for comparisons, with the methods already described (Section II). Crosses having been made both within and between lines it has been possible to compare the two groups in regard to seed setting. All pollinations were made by one person, who did not know whether she was »crossing» or »selfing». There is a difference in seed setting in favour of inter-line crossing with a P-value between 0,001 and 0,01.

Table 6 gives the results from weighings and measurements on the ripe plants; at the bottom of the table the degrees of significance of some differences are indicated. *Ert a* 19 and *ert a* 21 being alleles it is of interest to note the significant difference in plant height between them. There are also significant differences in ear density, number of florets per ear and ear length.

F_1 between the two mutant alleles is intermediate or nearly similar to either parent in individual characteristics. Both F_1's between mutant and Bonus are superior to both parents in tillering—number of straws

TABLE 6. *Studies on diallel crosses between Bonus, ert a 19 and ert a 21.*

Comb.	Number of			Length of		Weight of		Ear density
	plants	ears	florets per ear	straw	ear	plant	grain	
1. Bonus × Bonus	119	6,6	27,7	67	10,3	15,5	8,5	34,9
		± 0,26	± 0,22	± 0,53	± 0,10	± 0,74	± 0,42	± 0,16
2. 19 × 19	174	5,7	29,9	61	7,2	12,6	6,8	22,6
		± 0,29	± 0,31	± 0,35	± 0,08	± 0,76	± 0,44	± 0,17
3. 21 × 21	178	6,1	27,3	64	7,6	13,2	7,1	25,5
		± 0,19	± 0,20	± 0,50	± 0,06	± 0,52	± 0,30	± 0,11
4. 19 × Bonus	180	7,7	29,7	66	10,3	19,2	10,8	33,4
		± 0,25	± 0,19	± 0,49	± 0,08	± 0,70	± 0,39	± 0,12
5. 21 × Bonus	177	8,0	28,6	68	10,2	18,9	10,1	33,5
		± 0,26	± 0,20	± 0,54	± 0,08	± 0,69	± 0,37	± 0,11
6. 19 × 21	245	6,0	28,8	61	7,5	13,3	6,6	23,9
		± 0,18	± 0,21	± 0,51	± 0,06	± 0,48	± 0,26	± 0,09
Difference 1—2	t=		***	***		***		
» 2—3	t=		***	$3,41^{***}$		$3,27^{**}$		***
» 1—4	t=	$3,04^{**}$	***	0		$3,64^{***}$	$3,98^{***}$	***
» 1—5	t=	$3,82^{***}$	$3,00^{**}$			$3,32^{***}$	$2,90^{**}$	***

with ripe ears. In length of straw and ear, however, they are equal to Bonus, a result which does not agree with those of NYBOM, who found superdominance in these characters. *Ert a* 19 has a significantly larger number of florets per ear than Bonus and in this character F_1 shows full dominance for *ert a* 19. In combination with the superior tillering of F_1 this results in a superiority for the hybrid in total plant weight and yield of grain per plant. In the cross *ert a* 21 × Bonus F_1 has a larger number of florets per ear than both parents and also in this combination there is heterosis in plant weight and yield of grain. The length of internode in the ear is less in both F_1's than in Bonus, but the values are much closer to those of Bonus than to those of the homozygous mutants. There is no superdominance in regard to internode length or ear density (*cf.* NYBOM, *l. c.*).

V. DIALLEL CROSSES BETWEEN ERECTOIDES MUTATIONS. DIHYBRIDS.

The crosses made for the study of allelism and linkage of the ert genes (HAGBERG, NYBOM and GUSTAFSSON, 1952) have also been used for a direct study of the relations between hybrids and parents. Most

TABLE 7. *Data from the F_1 generation of diallel crosses between G-ert. 1949.*

Comb.	Number of plants	Number of ears	Number of florets per ear	Length of straw	Length of ear	Weight of plant	Weight of grain	Density of ear; internode length
Gull	274	4,85	27,9	82	9,4	9,8	4,3	34,7
G × loc. b alleles	53	4,23	28,9	82	9,5	10,1	4,1	34,2
G × other G-ert	88	4,59	27,8	79	8,9	9,6	4,1	32,6
G × all G-ert	141	4,45	28,2	80	9,1	9,8	4,1	33,3
loc b × other ert	101	3,95	30,0	82	9,4	10,4	3,7	32,9*
»other» × »other» ...	270	4,36	28,6	79	8,8	10,0	3,8	31,7
all G-ert × all G-ert ..	371	4,25	28,9	80	9,0	10,1	3,8	32,1
1 × other G-ert	56	4,38	27,8	78	8,8	8,8	3,6	32,0*
9 × » »	92	4,74	29,0	82	9,0	11,3	4,4	31,7*
7 × » »	51	4,08	28,8	76	8,7	9,4	3,5	31,6*
6 × » »	46	3,76	27,9	78	8,8	9,6	3,3	32,0*
11 × » »	25	4,64	29,1	80	8,7	9,8	4,4	31,1*
2	147	2,94	29,5	73	7,7	7,2	2,5	26,5
4	98	2,90	29,3	78	7,9	7,2	2,5	26,2
5	91	2,79	29,2	77	7,9	6,4	2,2	25,9
9	105	3,52	27,6	73	7,0	6,5	2,6	23,3
1	135	4,42	27,7	75	7,6	8,8	3,9	26,0
7	137	4,28	28,6	80	7,4	9,4	3,7	23,9
6	139	4,47	28,3	77	6,2	9,2	3,9	19,8
11	82	4,12	28,9	76	6,2	9,1	3,6	19,3

extensively studied are the mutants from Gull (G-erts, in 1949 and 1950) and the B-erts and some combinations between G- and B-erts (in 1951).

Of the G-erts the alleles 2, 4 and 5 in locus *b* have several characters in common, at the same time differing from the other mutants, especially in regard to ear density. In the characters here studied there are no significant differences between the alleles and they react in a similar manner to other ert genes; the data concerning them have therefore been pooled in Table 7. The most interesting data in this table and in Table 8 are those offering comparisons between the normal homozygote, *AABB*, monofactorial hybrids, *AaBB* or *AABb*, and dihybrids, *AaBb* (see also Table 2).

The alleles of locus *b* cause, as homozygotes and as heterozygotes, a poorer tillering than other ert genes. Tables 7 and 8 further show that the tillering, as a rule, decreases to some extent with an increasing number of ert genes. The same is true in the case of length of straw and ear and also in regard to length of the ear internode. In these latter

TABLE 8. *Data from measurements on F_1 of diallel crosses between B-ert and G-ert. 1951.*

Biotype	Number of plants	Number of ear per pl.	Length of straw	Length of ear	Density of ear; internode length
Bonus	403	4,6	62,0	8,9	32,0
Bonus × B-ert	120	3,9	59,7	8,3	29,9
B-ert × B-ert	577	3,6	57,3	7,8	28,7
Locus a × other B-ert:					
19 × other B-ert. loci	39	4,3	57,7	8,1	29,4
21 × » » »	162	3,2	57,7	7,9	28,7
23 × » » »	76	3,3	55,4	7,6	28,3
28 × » » »	60	3,6	56,4	7,7	28,2
Loc. a × loc. a	165	3,6	55,2	6,1	21,8
17 × other B-ert. loci	120	3,7	58,8	8,0	29,1
18 × » » »	223	3,6	59,9	7,8	27,9
24 × » » »	55	3,7	52,0	8,1	30,0
25 × » » »	81	3,2	56,0	7,7	29,2
27 × » » »	66	3,1	54,3	7,3	27,5
31 × » » »	91	3,7	56,0	7,9	29,2
32 × » » »	98	3,4	55,1	7,4	28,1
Gull	143	4,7	75,0	7,5	31,4
Bonus × Gull	138	4,6	75,3	8,2	31,8
Bonus × G-ert	46	4,1	70,3	7,8	28,9
B-ert × G-ert	590	3,8	65,3	7,3	27,9
1 × B-ert. loci	132	3,8	66,1	7,4	28,2
2 × » »	137	3,7	65,5	7,4	28,0
7 × » »	106	3,9	64,0	7,2	27,4
9 × » »	141	3,8	66,2	7,4	27,8
11 × » »	74	3,6	64,0	7,2	27,8

characters the mutants in locus *b* differ less from normal than the other erectoides mutants.

It has been found impossible to distinguish *ert* 20 from Bonus in any of the characters here studied; this mutant is not included in Table 8 and the »mutation» is on the whole dubious.

The total plant weights show that the monohybrids and still more the dihybrids have a tendency towards higher vegetative production than Gull, but the differences are not significant. In yield of grain per plant the mother line is superior to the hybrids. The number of florets per ear is, as a rule, high in the hybrids, and in combinations with locus *b* significantly higher than in Gull. The same was the case with *ert a* 19 and *ert a* 21, as shown already by Table 6.

TABLE 9. *Analysis of variance of internode length data in Table 7.*

Cause of variation	Sum of squares	DF.	Variance	Quotient
Total variation	1662,195	368	—	—
Between ertcombinations	203,558	7	29,08	7,20***
Between locus b and the other erts ..	149,647	1	149,647	37,04***
Between erts in locus b	22,964	2	11,48	2,84°
Between other erts	30,947	4	7,74	1,92°
Within combinations	1458,637	361	4,04	—

The length of the internodes in the ear is probably the character, among those studied here, which is the least influenced by environment, and where the standard errors are, therefore, comparatively small. The value for Gull is 34,70 mm ± 0,154. Monohybrid heterozygotes between Gull and G-ert have the value 33,32 ± 0,194; t for the difference is 5,57 and $P < 0{,}001$. Dihybrid heterozygotes between Gull and G-ert have the value 32,10 ± 0,111, and the difference between mono- and dihybrids has a t value of 5,46 and $P < 0{,}001$. The value for Gull × loc *b* is 34,17 ± 0,258, that for Gull × other G-erts is 32,57 ± 0,249; the difference has $t = 4{,}46$ and $P < 0{,}001$. An analysis of variance of the values for length of ear internode in dihybrid combinations (marked with an * in Table 7) is found in Table 9. Among the dihybrid heterozygotes those including one allele from locus *b* are significantly more lax eared than the others; between these or between the alleles in locus *b* there are no significant differences.

Finally a few words may be added about the differences between different ert mutations in regard to the relation between homozygous and heterozygous states in their effect on ear density. There is no definite correlation between the two effects, which is most easily seen from the following figures, giving the data for four different B-erts, when the internode length figure for Bonus itself was 32,0.

Ert-gene	Internode length values for		
	aa	*Aa*	dihybrid combinations with B-erts
17	15,3	30,6	29,1
18	26,4	28,9	27,9
24	22,7	32,4	30,0
25	28,8	31,2	29,2

There are some indications that the heterozygote of *ert a* 21 should have a more lax ear than those of other alleles in locus *a*.

In the summer of 1952 some tetrahybrid combinations have been grown. The measurements are not all made at the date of writing, but some preliminary data may be given. Plants heterozygous for *ert c* 1, *b* 5, 9 and *a* 11 have the value 24,1 as corresponding to about 33 for Gull, and heterozygotes for *ert b* 4, *a* 6, *d* 7 and 9 have the value 25,4.

VI. DISCUSSION.

The theory of JONES (1917) explaining heterosis as caused by the combination in the heterozygote of a large number of dominant factors, many of them necessarily linked, has for a long time been accepted by most authors. This theory also certainly gives an essential part of the truth about heterosis— but perhaps not all of it. FISHER (1918) points to the possibility of what he calls superdominance ($Aa > AA$). For the same phenomenon HULL (1949) uses the term overdominance. JONES (1921) has presented a material of *Nicotiana* with monohybrid segregation where the heterozygotes are taller than both homozygotes. JONES writes: »Here, apparently, is what might be taken as an instance of hybrid vigor where the parents differ in only a single gene» ... »But there is no hybrid vigor shown in so far as this is represented by an accelerated rate of development». More than twenty years later, JONES (1945) presented a second contribution to the discussion of monofactorial heterosis. QUINBY and KARPER (1946, 1948) describe a case of monofactorial heterosis in *Sorghum*, which is partly similar to JONES's case in *Nicotiana*. In a series of papers the dominance and superdominance theories have been discussed (*e. g.*, EAST, 1936; CROW, 1948; HENDERSON, 1949; BRIEGER, 1950, *etc.*). STUBBE and PIRSCHLE (1940) presented a case of superdominance, as the term is limited by NYBOM (1950). They demonstrated a higher content of chlorophyll than in normals in plants of *Antirrhinum majus*, heterozygous in a chlorina mutation. MÜNTZING (1945) maintains that this case cannot be taken as a proof of »stimulation» (superdominance), since the mutation may in reality not be a »point mutation». This criticism seems to be valid in all cases where spontaneous or induced mutants are compared with the parent strains, since it seems to be difficult, even impossible, to prove definitely that any given mutation is not more or less complex. For such a proof we would need a far deeper knowledge of the nature of the genes than we have at present. BRÜCHER (1943) has shown that the heterozygote of an induced semilethal mutation in *Antirrhinum*

»zeigte ein ausgezeichnetes Wachstum, welches bei bestimmten künstlichen Umweltbedingungen das der Normalform um ein Mehrfaches übertraf».

STERN and NOVITSKY (1948) and STERN *et al.* (1952) state that induced X-chromosome lethals in *Drosophila melanogaster*, as a rule, cause a decrease in viability also in the heterozygous state, but there are exceptions also in STERN's material. As already mentioned, a number of homozygously lethal chlorophyll mutations have been studied by GUSTAFSSON and his co-workers in 1946—1951 and in several cases these mutations increase the vigour in their heterozygous state.

In his paper on superdominance in erectoides mutants, NYBOM (1950) discusses the terminology and attempts a distinction between superdominance and monohybrid heterosis. He restricts superdominance to those cases in which the heterozygote is superior to both homozygotes in regard to the specific character influenced by the gene in question, while monohybrid heterosis should mean that the heterozygote is superior in some character concerning »general vitality», such as plant height, tillering, production, *etc.* The »erectoides-genes» studied by NYBOM and the present author are, however, pleiotropic in their effect upon the phenotype, and, thus, it becomes difficult to maintain NYBOM's distinction.

The pleiotropic effect of the genes also sometimes makes it difficult to decide whether they should be said to be dominant or recessive. If the definition of dominance, given by the Nomenclature Committee (LITTLE, 1921) is accepted, *ert f* 18 is dominant in ear density but recessive in length of straw. The heterozygote of *ert a* 19 is intermediate in ear density but much closer to the normal type, and the mutant must be classified as recessive in this respect. In total weight of plant the normal allele is superdominant but in regard to the number of florets per ear the mutant allele is completely dominant and significantly different from the inferior normal allele. Whether the mutant allele is designated *a* and the normal one *A* is, in many cases, a matter of which of the effects is considered the most important one or, simply, was first studied. So-called recessive mutations may easily be dominant in their effects on some characters of the individual.

The occurrence of multiple alleles in certain loci and especially the fact that the »*a*» allele has a definite influence in the heterozygote may be taken as a positive proof that the differences between normal homozygotes, heterozygotes and homozygous mutants are not only due to the presence of 2, 1 or 0 *A* factors. As an example may be taken the

number of florets per ear in the combinations *ert a* 19 × Bonus and *ert a* 21 × Bonus. If the heterozygote is compared with the homozygous mutant it is found that the effect of the introduction of one *A* allele in otherwise identical gene complements is significantly influenced by the remaining »*a*» allele, whether it be $a_{a\,19}$ or $a_{a\,21}$.

In this connection CASTLE's (1946) hypothesis should be mentioned, that superdominance is caused by *A*/*a*, while in other cases the heterozygote is *A*/—, »the — representing the condition of that locus in the chromatin prior to the origin of the *A* gene». Since *ert a* 19 is recessive in its effect upon ear density but dominant in its effect upon the number of florets and the normal allele in this combination superdominant in regard to total plant weight it is hardly possible that the occurrence of superdominance is due to the existence of a recessive allele, while its non-occurrence should indicate the »absence» of any active allele.

Since the mutations — genes — have pleiotropic effects with varying dominance in different part-effects, it might be suitable to symbolize the phenotypic effects of one mutant allele with *aBc*, *etc.*, the effects of the corresponding normal allele with *AbC*, *etc.* It then becomes quite evident that the heterozygote may give a better phenotypical result than any of the homozygotes.

We are only able to study the phenotypical end result of the effects of a gene. The gene may possibly have only one single primary effect on the basic enzymatical processes of the nucleus, but this primary effect may easily influence a number of characters. The effects of a mutant gene may be favourable or adverse. Two alleles may be complementary, just as the whole genotypes of two inbreds complete one another, and the heterozygote may be better suited to give a »phenotypical response» to different environments. *Aa* should, in other words, be better able to utilize the resources available for the production of a vigorous individual of high competitive value. This most probably is not always the case, but it may be true in a number of combinations of alleles. It is the author's intention to discuss these problems more in detail in a comprehensive summary of his work on the heterosis problem.

SUMMARY.

Erectoides mutants analysed in regard to their location are designated with a letter for the locus and their original number, *e. g.*, *ert a* 6, *ert a* 11, *ert a* 13, *etc.* Another series is *ert b* 2, *ert b* 4, *ert b* 5, *etc.* Fourteen different loci have so far been determined. The segregation

of several mutants has been studied; the proportions are fairly close to 3 : 1 with a significant deficit in recessives.

Of seven extensively studied combinations mutant × mother line two showed superdominance in total plant weight, yield of grain per plant and tillering, one of them also in number of florets per ear. The relations between the different genotypes in segregating material in regard to vigour seems to vary from year to year.

Comparisons between mono- and dihybrid heterozygotes and normals show that the effects of the mutant genes in many cases are cumulative; such is the case with ear density, the most extensively studied character.

The mutations are pleiotropic in their effect upon the phenotype. In some of these effects the mutant allele may be recessive, in another it may be dominant and in regard to a third effect the normal allele may show superdominance. The mutant gene may have both positive and negative part-effects upon the phenotype, and two alleles seem sometimes to be able to act as complements to each other, thus causing monofactorial heterosis.

Svalöf in August, 1952.

When this paper already was written the author received the volume on the Heterosis Conference in Ames, Iowa, in 1950, (GOWEN, 1952). It has, unfortunately, been impossible to work all the interesting data and viewpoints presented in this book into the discussion here, but the author hopes to return to them in a following paper.

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STUDIES OF DISEASES IN CLONES OF FOREST TREES

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IN forest tree breeding one might often be inclined to follow exactly the lines whereby agriculture and horticulture have obtained such splendid results. This would seem natural enough, as in all cases living plants are concerned. There are, however, in reality such considerable differences that it may be important to take these in consideration at an early stage. First and foremost, the forest trees are individuals of considerable longevity, and harvesting of a stand takes place at different periods, the greatest being of course the final cutting. In intensive forestry, however, an almost equal volume should be obtained by the repeated thinnings.

On account of the long rotation, the aim of all forest tree improvement must be to secure resistance of the stand to disease, whilst the claim of similarity may be more relaxed. We may harvest the individual tree by a thinning or let it remain with the main crop, the farmer must harvest his crop collectively at the appropriate time. He may, however, from one year to another change his cultivated strain on learning that a certain strain is strongly prone to disease. This the forester cannot do. He will suffer a great loss if forced to cut a stand before it has attained maturity.

One may therefore maintain that, at any rate, an immediate aim of forest tree improvement should be to strive for a uniformity of the stands free from inferior specimens, whether with regard to resistance to disease, growth, or form.

The form of a tree is prominent to the eye and it follows that considerable attention should be paid to it, but it must not be forgotten that certain possibilities exist of an improvement of the form through appropriate treatment, whilst this is not the case with growth. The result of the latter may be connected with resistance. These three factors, as well as other individual qualities, we have a chance of estimating through »tree-shows».

By the estimation of a series of individuals in a tree-show, one might become enthusiastic about a certain clone. One might consider

that it possesses all desirable qualities and be tempted to test it further and grow it by vegetative propagation. Such employment of the clone may seem so inviting that, in intensive forestry, it may seem the only reasonable course to take. One will be taking a chance, the reward, however, of a successful outcome may be so great that one is willing to pay the greater price for the lay-out of the culture, taking the risk of failure.

On the whole, I would advise against too close a selection as long as the limited knowledge of the qualities of the individuals remains.

When selecting a thoroughly tested clone for cultivation, we may obtain the greatest possible safeguard from the beginning. Not only should the development of the clone have been tested in comparison with other clones subjected to similar conditions, but in relation to different external conditions. It is only possible, however, to subject it to such external conditions as are known today. Others may conceivably present themselves by the appearance of new diseases, or one at present unknown, as has occurred in agriculture and horticulture.

In other cultivation it has been feasible to take the risk of using clones, since it is possible to change from one clone to another. Also extensive means of checking disease by chemical means may be used. Comparable means may find further use in forestry and become of special importance in countering some acute attack which may have been favoured by environment.

The effect of a specific chemical treatment may be so strong as to completely eliminate the danger, but it may also act as a powerful one-way factor which, in a somewhat similar way as in our improvement schemes, forces development in a certain — and unwanted — direction. It is to be hoped that we may still find new methods of countering such eventualities. It will always, however, present a financial burden, and possibly a danger which can be met with only when there is a genuine prospect of ultimate profit.

An example of the cultivation of clone and population within the same genus is found in the cultivation of poplar and aspen (the section Leuce Duby). To a certain degree this also applies to larch and thuja at the present stage of Danish forest tree improvement. The use of clones of poplar with its advantages and disadvantages is generally known. When the Swedes first, followed immediately by ourselves, obtained very promising hybrids by a cross of European (*Populus tremula* L.) with North American aspen (*P. tremuloides* MICHX.), it was tempting to follow the method employed for poplar and go in energetic-

ally for selection and cultivation of clones. With very promising offspring, the variation is so great that such a procedure might seem reasonable. It could be done, and certain advantages might be gained. In Denmark, however, we believe that the best results may be obtained by an annually repeated production of seedlings.

The seed is produced in greenhouse by controlled pollination between the selected breeding trees. In this way offspring of high volume production are obtained. Simultaneously, the considerable variation of the seedlings increasingly safeguards them against diseases. One may also reap the benefit of the characteristic conditions appertaining to forest cultivation insofar as only a small proportion of the plants set out is retained in the main crop.

Whether now we attempt using the individual trees for cultivation in clones, or we choose a greater or smaller number of individuals for utilization in seed-gardens, it must be a condition that such trees are known to us through an estimation in »tree-shows». We must not depend only on the phenotype such as we find the trees in the forests often great distances apart and subjected to varying external conditions.

Such tree-shows have been established at the Arboretum of the Royal Veterinary and Agricultural College at Hörsholm from grafts made from 1935 onwards. The earliest ones chiefly comprise ash and beech as well as larch and Norway spruce. Our tree-shows of Douglas fir of similar age were considerably extended in 1946 with selected trees from the original stands on the West Coast of North America. The scions were sent by air mail. This has proved invaluable in carrying living material such as scions and pollen.

In 1947 (SYRACH LARSEN, 1947) I drew attention to the value of tree-shows in forest tree improvement. In a small article published later (SYRACH LARSEN, 1951) I enlarged on this idea as the means to attempt to follow the individual tree »from the grave to the cradle», and now I would like to give a few examples of their importance in the fight against diseases.

It is quite usual to hear the remark that in reality we know nothing of a plant until we have been able to follow its development through the whole of its life-time, »from the cradle to the grave». Such remarks are very natural, since it can be said to form the basis for all agricultural improvement. If, however, we had to wait similarly in forestry, the time taken would be so great that it would be difficult to overlook the problems. We must, therefore, make use of every possible short cut

in order to arrive at the correct exposition of the problems of our work. May it not be that the farmer follows his plants from the cradle to the grave because it is so easy? Is it not the fact that he has to produce true breeds because his plants are so short-lived? Were he to find an excellent individual with, for instance, high resistance, would he not want to preserve it and make it the basis of annual cultivation in clones or breeding plants? Likewise, he would no doubt be delighted at the easy preservation of other good individuals when they had been proved by the »poly-cross» method to be possessed of high »combining ability»? All this he would find difficult, and we lack his advantages in many ways, whereas we by our method may profit by the longer living individuals.

From a »tree-show» we gain the technical help to undertake, at any rate, a partial *analysis* of the individual. Our older trees, long subjected to their often varying environment, may be presumed to show particularly great phenotypical influence. It is difficult to undertake a direct comparison between the individuals grown in a free position and those grown in close stands; there may be many other variations in the external conditions. Furthermore, we have little possibility of judging the external conditions prevailing in the early years of the tree, and we cannot transplant the old trees, themselves.

As it is difficult for us to judge the individuals built up through many years, it is not easy to obtain an estimate of the progeny requiring a similarly long time to develop. Thus, if our forest tree breeding is to have any hope of comparatively speedy development, we must endeavour, to a certain degree, to free ourselves of numerous progeny tests. We must do this by going back as far as possible in an analysis of individuals which have proved, perhaps during 50—100 years, to be of value. We must search for the outstanding individuals and include them in our experiments, and it is my opinion that with the help of vegetative propagation, we may be able to subject them to such examination that we may get an explanation of the conditions which have caused their predominance.

A »tree-show» consisting of grafts, possibly buddings, or cuttings, may comparatively quickly tell us whether the individuals examined possess any special qualifications, one way or another. It may be hardiness, form of growth, resistance to disease, or the, in my opinion, very important growth vigour. After a simple comparison of the clones under similar conditions, we may proceed to a testing of the various clones. This may be done by setting out on different areas, or by arti-

ficial testing at research stations. Though somewhat feebly, we may thus approach the chemical engineers analysis of the present with a view to thereby being able to produce our desire.

By such methods plant improvement in forestry would compare, to some extent, with the work of the engineer. He must depend, to a great extent, on detailed experiments, often only dealing with a part of the work in order to calculate his way towards his goal. He cannot make the great building constructions by experiment alone. He must know beforehand a wealth of details on which to base his calculations. A tree is also a building construction placed on one spot for 50—100 years. I am aware, of course, that such comparison does not quite cover the case, but there are important similarities.

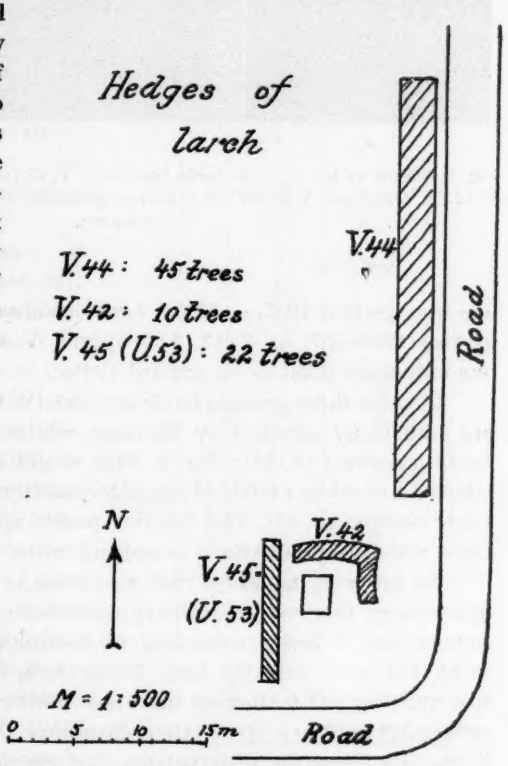
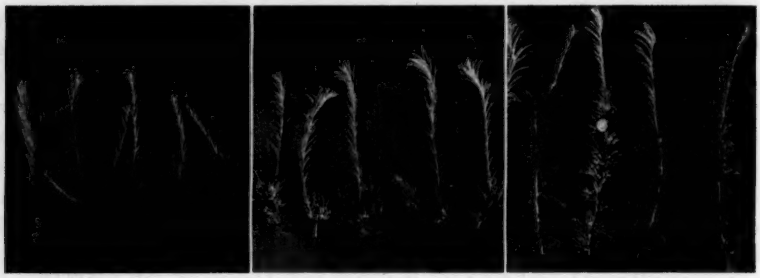


Fig. 1. See text.

By use of grafts where, at any rate, the parts above the ground are genetically identical, not a few valuable details may be obtained concerning the future employment of the single individuals. It is, *e. g.*, easy to work with larch, Douglas fir and thuja, of which a few examples may be mentioned. Larch has been worked with for the longest time; Douglas fir is of particular interest because of the two types, »blue» and »green», each subject to its peculiar enemy, and thuja is easily propagated in clones by aid of cuttings.

The situation as regards larch is shown in Fig. 1. We have here three hedges of clones of larch: V. 45. *Larix leptolepis*, 22 grafts from



a *b* *c*

Fig. 2. Shoots of larch. — *a. Larix leptolepis*, V. 45 (no *Chermes*). — *b. L. decidua*, V. 44×*L. leptolepis*, V. 45=V. 42 (*Chermes* present). — *c. L. decidua*, V. 44 (*Chermes* present).

one tree, grafted 1936. — V. 44. *Larix decidua*, 45 grafts from one tree, grafted 1936—37. — V. 42. The hybrid, V. 45×V. 44, 10 grafts from one individual plant in F_1, grafted 1936.

Of these three groups, *Larix decidua* (V. 44) and the hybrid (V. 42) are both badly attacked by *Chermes*, whilst *Chermes* is not found on *Larix leptolepis* (V. 45); Fig. 2. This would show clearly that the one selected hybrid has retained the susceptibility to *Chermes* of the father, *Larix decidua* (V. 44). This inferior quality appears on all plants of this clone which is, otherwise, a promising cross.

To this may be added that a number of sisters of V. 42, given an opportunity to grow as ordinary trees have shown no attacks of any importance. A hedge consisting of seedlings from the same species-cross, but with only the same father tree, has however shown great susceptibility to *Chermes* on the whole. This may indicate that trimming as a hedge may to a great extent encourage *Chermes*. At the same time, it appears from the observations that the individual used (V. 45) of *Larix leptolepis* has proved resistance to *Chermes*.

Another example of an attack on a clone of larch by insects may be mentioned. In the spring of 1943 a few rows of clones were grafted, among which were three clones of Scotch larch selected from a Danish provenance test. Of these, three clones were grafted each a row of seven specimens. During the first years they all developed without any external damage, but in the summer of 1946 one clone was attacked by a moth, *Argyresthia laevigatella* H. S., damaging the top shoots. It looked very strange that every leader in this clone was broken (clone

V. 629), whilst not a single one was damaged in the two adjoining rows containing the other two clones (V. 628 and V. 630).

As regards *Pseudotsugo taxifolia* (POIR) BRITT. we have, amongst others, two clones grafted in the spring of 1946 and set out as shown in Fig. 3. V. 1049, grafts from a tree by Gold River, Vancouver Island. There are five plants of the following heights, respectively: 1,80, 1,80, 1,50, 1,85, and 1,90, averaging 1,77 m. — V. 1063, grafts from a tree by Wind River, Washington. There are six plants of the following heights, respectively: 2,05, 2,90, 2,80, 2,95, and 2,90; averaging 2,73 m.

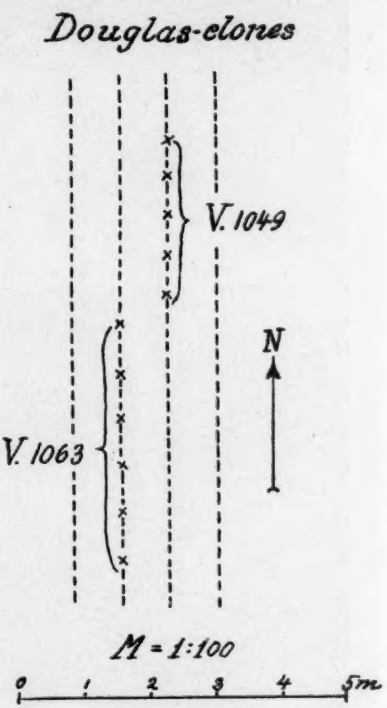


Fig. 3. See text.

Besides the difference of height it may be mentioned that V. 1049 is of a fine and uniform growth, whilst the plants in V. 1063 are all winding and of unfortunate form as regards the rather exaggerated leaders. To this should be added, what is most important in this particular case, *i.e.* all five plants of V. 1049 are severely attacked by *Chermes Cooleyi* GILLETTE, while *Chermes* is not found on any of the six plants of V. 1063 (Fig. 4). Both groups have been here in the Arboretum since the scions arrived in the spring of 1946 directly from trees in the natural stands. They have, furthermore, been so long in their present position in proximity to other clones of Douglas fir, more or less attacked by *Chermes Cooleyi*, that the danger of infection should be equal to both.

When *Chermes Cooleyi* attacks single individuals in our stands to a varying degree, it seems as if the above little experiment would justify the presumption that the attacks are, to some extent, determined by hereditary tendencies. The badly attacked clones should therefore not be included in our seed-gardens.

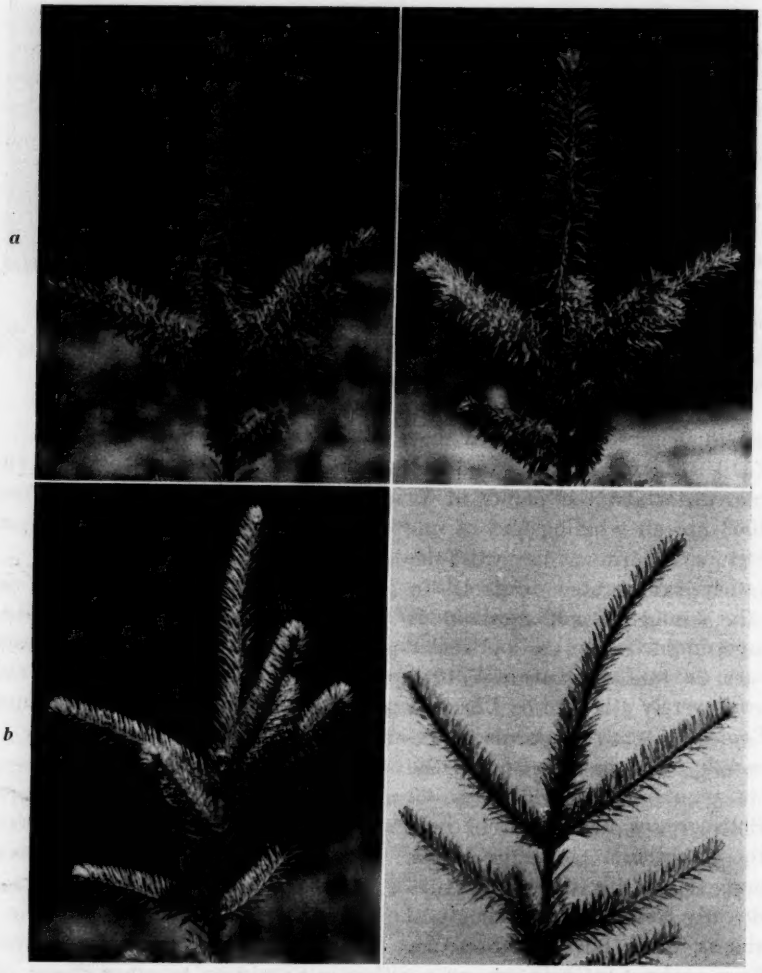


Fig. 4. Top shoots of *Pseudotsuga taxifolia*. — *a*. V. 1049, grafted 1946 (*Chermes* present). — *b*. V. 1063, grafted 1946 (no *Chermes*).

On a somewhat older experimental area where we have grafted clones of Douglas fir since 1943, we have started work in a stand of about eight years consisting of a mixture of greatly varying forms from the most green and prolific to the grey and slow-growing individuals.

There were pronounced signs of attack by *Rhabdocline pseudotsugae* SYD. and *Phaeocryptopus Gäumanni* ROHDE, the former keeping to the blue and grey forms, whereas the latter is a danger to the green Douglas fir, in this country anyhow.

By the presence of these two fungi one would be able to test on the spot the clones of Douglas fir used for the grafts. No cases of *Chermes Cooleyi* were observed in the area at the start of the test. This pest was first observed in Denmark in 1938 (THOMSEN, 1940), but later it has spread considerably in the testing area.

DOW V. BAXTER, Ann Arbor, Michigan, visited the area in the summer of 1950, on this occasion taking the photograph (Fig. 5) used as Fig. 178 in his »Pathology in Forest Practice» (BAXTER, 1952). His figure shows a graft (V. 661) so far healthy six years after being grafted next to one of the original non-grafted plants severely attacked by *Adelophus* (*Phaeocryptopus*) *Gäumanni*. All the trees of the clone V. 661 are still untouched by *Phaeocryptopus* as well as *Rhabdocline* here where every opportunity for infection has existed through many years.

Fig. 5. *Pseudotsuga taxifolia.* — Right: V. 661, grafted 1946. — Left: Original seed plant.

The case of *Chermes Cooleyi* appears to be somewhat different. Clone V. 661 is not resistant to *Chermes*, which is present, but the trees seem unaffected by it. During the first years after the stand first being attacked, we noticed only weak attacks in V. 661. Last summer (1952) was a very pronounced *Chermes* year, also as regards *Chermes Cooleyi*. Consequently, V. 661, was badly infected by *Chermes*, but still seemingly

vigorous of development, thereby differing from other clones which show a distinct weakening after the attacks.

This case shows that it may be of importance to have fungi and other pests within the country's borders in order to get an opportunity to judge the reaction of the individuals to such attacks. It may be possible, of course, to slow down such attacks by plant control, at least for a time, and it would not be advisable actually to import the diseases. At the same time, it would not be amiss to realize that such early preliminary testing may well help to safeguard against the further spreading of the disease. The forester should not in desparation reject a new and promising type after the first, perhaps violent, onslaught of a disease.

For the above-mentioned test of Douglas fir only clones from trees promising to be of forestal use were grafted. Thus, no blue or grey varieties were included, and none of the green ones were attacked by *Rhabdocline pseudotsugae*.

The question also presents itself whether or not the root or the part of the original tree below the union, may, to some degree, influence the development of the graft. For instance, would a *Larix decidua* MILL. severely attacked by *Dasyscypha Willkommii* HTG. be more healthy if it were grafted on *Larix leptolepis* (SIEB. et ZUCC.) GORD. which appears to be resistant to the fungus? This is a question I have been asked, but have been unable to answer.

Amongst our experiments with Douglas fir in the Arboretum we have, however, an example which may, to some extent, elucidate the question.

A 20 m specimen of *Pseudotsuga taxifolia glauca* (MAYR.) SCHNEID. is found in the Forest Botanical Garden at Charlottenlund, an older arboretum which also forms a part of the Royal Veterinary and Agricultural College. This tree was planted in 1889 and distinguishes itself from other specimens of the type in this country by its prolific growth without a trace of *Rhabdocline pseudotsugae*, in spite of the fact that the disease has been present in the garden during the last 15—20 years.

Only about 50 m apart is a 28-year-old Douglas fir of the blue-grey type from Yellowstone National Park, Wyoming, which over the whole period has been constantly and strongly attacked by the fungus. The old and vigorous tree might therefore be described as resistant to *Rhabdocline pseudotsugae*. Experiments with this tree in a tree-show of only a few grafts give, however, a somewhat different picture.

On vigorous, typically green Douglas fir, planted 5 years earlier,

Fig. 6. *Pseudotsuga taxifolia*, blue (*a*) (*Rhabdocline* present), grafted 1943 on five-year-old *Pseudotsuga taxifolia*, green (*b*) (no *Rhabdocline*).

were grafted in May, 1943, a few scions from the above told old *Pseudotsuga taxifolia glauca*. During the first years after grafting, the scions developed into vigorous, typically blue and wholly sound new specimens, which gave the appearance of small blue trees on the top of green Douglas firs which had been cut down to about 1 m above ground. After a few years continued favourable growth, they were suddenly and severely attacked by *Rhabdocline pseudotsugae*. The attack has repeated itself every year since to an equal degree on all four grafted specimens which together make a little tree-show. Towards the end of summer, they appear almost entirely devoid of needles excepting the being year's shoot, thus presenting the characteristic symptoms of *Rhabdocline* (Fig. 6).

There is no sign whatever of the disease being more easily overcome because of the grafting on virulent green Douglas fir. Besides allowing the blue Douglas fir grafts to develop freely and retain all side

shoots, the branches of the stock have also been retained in order to make the stems as vigorous as possible, and to obtain the best possible contrast between the two types of the same species.

As I have endeavoured to show on Fig. 6, the blue Douglas fir now appears as diseased upper parts on a vigorous lower part of typically green Douglas fir which, characteristically enough, are attacked by a certain amount of *Chermes Cooleyi*, but not by *Rhabdocline pseudotsugae*. On the other hand, the blue tops, ravished by *Rhabdocline*, show no sign of a single *Chermes Cooleyi*. This makes an exceptionally sharp distinction which does not allow the supposition that the two parts of the trees are influenced by one another. This is in agreement with other observations made in the Arboretum. In spite of a great many grafts on widely varying populations which have been used as stocks, the grafts within the clone have an equal development. There may, of course, be influences which we are unable to observe, but so far, the considerable number of tree-shows here seem to develop in a remarkably uniform way. One way or another, it would seem as if really great differences between the scion and the stock were required in order to produce the diversities at present recognizable.

We must return, however, to the old *Pseudotsuga taxifolia glauca*. Why is this tree not attacked by *Rhabdocline* when the disease has existed for several years in its immediate vicinity? This is so much more remarkable as the disease has for several years been rampant in Denmark and grafts from the same clone have been attacked in the Arboretum after a few years.

It may indicate that we have to show caution in regarding such an old tree as resistant even though it cannot be attacked in its present stage. It may be that the disease was non-existent during the early years of its growth in the Forest Botanical Garden. The disease was first discovered in Scotland in 1922, and in this country in 1929. Up till that time, we had not a few quite vigorous stands of blue Douglas fir in our forests, all of them however quite a bit younger than the tree in the Forest Botanical Garden. May it be that by its earlier start it has passed the dangerous age, thereby reaching such development in power and age as to be no longer susceptible.

If this should prove to be the answer, we must be cautious in our estimation of old trees of other species as well, which may have arisen under similar conditions.

As regards *Thuja plicata* LAMB. (Western Red Cedar), we have suffered for a number of years from unpleasant attacks of *Didymascella*

thujina (DUR.) MAIRE. Thuja is easy to work with as it is propagated by cuttings without any difficulty; thus, completely identical trees can easily be produced.

By using cuttings we have obtained some clones showing definite tendencies and also the value of the method for estimations of their importance in forestry. As worthy of special promise may be mentioned the clone V. 469, which we have planted in two small tests in the Arboretum, both places bearing cuttings from the spring of 1942. In the one case the clone is compared with contemporary cuttings from another clone (V. 470), while in the other comparison is made with seedlings sown at the time the cuttings were put into frames. The result is as follows:

1st test:		2nd test:	
Clone V. 469: Average h.	5,7 m	Clone V. 469: Average h.	5,7 m
» V. 470: » »	3,1 »	Seedlings: » »	3,9 »

As all the plants were started simultaneously and must be supposed to have had equal opportunities, the varying heights appear very conspicuous.

If we leave the seedlings out of comparison, as it is a question of different methods of propagation, and consider only the two clones mentioned, the following observations may be relevant, as regards the diverging developments: Clone V. 469 has all the time been untouched by frost and *Didymascella thujina*, whilst the other clone of poorer development, V. 470, has suffered from both, especially from frost.

The valuable clone, V. 469, derives from a tree in the Forest of Linå Vesterskov, in Jutland; the old tree does not present a conspicuous picture of strength and dimensions according to its age. It grows in a locality not very favourable for the species. Immediately following some particularly hard winters (1939—1942), however, it appeared as practically the only specimen in the locality of a fresh green colour to the very top, thus giving the impression of being possessed of unusual hardiness. Possibly it may be this quality which first and foremost determines the conspicuous growth of the clone. In this country, it is possible that the otherwise fairly severe attacks by the fungus on young and youngish thuja plants are primarily determined by a certain unsuitability of our climate, so that attacks of *Didymascella* may perhaps be termed of secondary importance.

The above argument in no way pretends to be capable of elucidating the diverse questions of the diseases mentioned, and the term

»disease» has been used broadly to include attacks by fungi, insects and frost. In practical forestry, and therefore also in forest tree breeding, the diverse questions are often distinguished only with difficulty, all remaining of equal importance.

Concerning plant pathology and forest entomology, further experiments will be required. In addition to laboratory tests and the »tree-shows», it would be advisable to undertake tests at the research stations.

Another question may here be touched upon. It is very important to note that besides the plants produced for tree-shows, a number may be grown for early fructification. By somewhat rough treatment one may often succeed in getting such vegetatively propagated plants to fructify at a surprisingly early stage.

In order to arrive at improvement more quickly and safely, we cannot do without outside help, *i. e.* from forest pathologists and entomologists. A wider co-operation is needed in one way or another. Only we foresters must not let ourselves be enticed too far from our own sphere. Rather should we try to encourage the pathologists and entomologists to visit our forests or research areas in order to study the »patients». By the aid of tree-shows — of the *long-living* individuals of the forest — we may even go so far as to collect the »patients» in order to present them all lined up in their »hospital beds». That would be the moment to call in the specialist, and we foresters should avoid dabbling on his field of operation.

Here in Denmark, our forestry has been favoured by the great interest shown by both the pathologists and the entomologists, and if this co-operation could be further extended, it would be of exceptional economical importance to forestry and would also, it is to be hoped, prove a great pleasure to the men who work with these problems.

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SEX LINKED LETHALS IN DROSOPHILA MELANOGASTER FROM DIFFERENT MODES OF X-RAY IRRADIATION

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I. INTRODUCTION.

IN a preceding study (BONNIER, LÜNING and ARNBERG, 1952) it was found that the rate of x-ray induced recessive lethals in the paternal X-chromosome of *Drosophila melanogaster* was higher if the irradiation was given to impregnated females than if it was given to males prior to insemination. The dose used in these experiments was 960 r and the figures found were 3,48 per cent recessive sex linked lethals in the former case and 2,04 per cent in the latter; the difference corresponded to a significance level of $P = 0{,}05$. As in that experiment the eggs were collected within 8 hours after irradiation, it was tentatively supposed that if fertilization takes place shortly after irradiation a mutagenic effect from the irradiated egg cytoplasm might be exerted upon the male chromosomes. It was, however, also stressed that further experiments were desirable, and such experiments are now performed.

II. TECHNIQUE.

All experiments presented in this section are made by crossing *y w sn* females to *M5* (= sc^{S1} *B In-S* w^a sc^{S1}) males after which the F_2 offspring from individual F_1 females were checked. As all cultures are raised by letting the females deposit their eggs on food slices attached to card board strips, which then are removed to vials with tissue paper soaked in yeast suspension, no parents are to be found among the hatching offspring. The male F_2 offspring consist of two types, *viz.* *y w sn* and *M5*. When an F_2 vial contains no *M5* males this indicates that the F_1 mother of that vial had a recessive lethal in her paternal X-chromosome. In those cases, however, where there are only very few *y w sn* males and no *M5* males it is, of course, uncertain whether this is a real case of lethality or if it is due to chance together with effects of the slower development of *M5* than of *y w sn* males. (In a recent paper by STERN, CARSON, KINST, NOVITSKY, and UPHOFF, 1952, the

difficulties involved in this fact are discussed at a considerable length.) In the previous article mentioned above (BONNIER, LÜNING and ARNBERG, 1952) we tried to overcome the difficulty by discarding all F_1 females in the F_2 progeny of which — if necessary added with an F_3 generation — there were less than 15 *y w sn* (and no *M5*) males. However, when at a later occasion going through our figures it was observed that if we had discarded less of the F_1 females there would have been found a smaller difference between the frequency of lethals after irradiation of the paternal X-chromosome in impregnated females as compared with the corresponding frequency after irradiation of males. In the present article we are, therefore, presenting the results in two ways: A, after discarding all F_1 females which in F_2 (and if necessary together with F_3) had no *M5* and 5 or less *y w sn* males; and B, after discarding of F_1 females for which the corresponding number of *y w sn* males was 9. Moreover, as it now and then happens that an F_2 culture contains many *y w sn*, together with very few *M5*, males, *i. e.* cases of semi lethality, we present the results for both of the A and B classifications in two ways, *viz.* on the one hand including the cases only which were classified as true lethals, and on the other hand these same cases to which were added the semi lethals. We must, however, refrain from giving a distinct definition of what we mean by a semi lethal. We simply classify such cases as semi lethals where, in the progeny of F_1 females, the number of *M5* males is very small in comparison to the number of *y w sn* males. It should also be mentioned that the vials containing the progeny of F_1 females were inspected without etherization: a vial in which one or more *M5* males were seen was reckoned as a non-lethal case (though in very few instances such a vial might have contained a semi lethal); if no *M5* males were seen etherization was undertaken and the progeny was carefully classified.

III. THE EXPERIMENTS.

If the tentative assumption mentioned in the introduction was correct it would follow that by irradiating impregnated females and collecting the eggs during different time intervals after irradiation, the percentage of lethals would decrease the longer after irradiation the eggs were laid. Therefore, in several cases the experiments were planned according to this assumption. So, for instance, in some series eggs were collected during the following time intervals after irradiation, 0—6, 6—24, and 24—48 hours; in other series during the intervals, 0—24,

TABLE 1. *The production of recessive lethals and semi lethals in the M5 X-chromosome after irradiating*

TABLE 1. *The production of recessive lethals and semi lethals in the M5 X-chromosome after irradiating spermatozoa with 960 r. The M5 males were mated to y w sn females and the irradiation was given either to impregnated females (F) or to males (M). The experiments were carried out at four different occasions (sets 1—4). F_1 females were classified as carrying a lethal if in their progeny there occurred no M5 males and at least 5 (A) or at least 9 (B) y w sn males. Cases where there in the progeny were very few M5 males as compared with the number of y w sn males were classified as semi lethals.*

Set	Mode of irradiation	A. Exclusion of F_2 vials containing no *M5* males and not more than 5 *y w sn* males					B. Exclusion of F_2 vials containing no *M5* males and not more than 9 *y w sn* males				
		Nr. of tested chromosomes	Nr. of lethals		Nr. of lethals + semi lethals		Nr. of tested chromosomes	Nr. of lethals		Nr. of lethals + semi lethals	
			Abs. Nr.	%	Abs. Nr.	%		Abs. Nr.	%	Abs. Nr.	%
1	F	844	29	3,44	31	3,67	844	29	3,44	31	3,67
	M	1531	45	2,94	50	3,27	1524	38	2,49	42	2,76
Diff. and sum		2375		0,50		0,40	2368		0,95		0,91
2	F	2700	78	2,89	79	2,93	2699	77	2,85	78	2,89
	M	1465	27	1,84	27	1,84	1465	27	1,84	27	1,84
Diff. and sum		4165		1,05		1,09	4164		1,01		1,05
3	F	3285	110	3,35	112	3,41	3279	104	3,17	106	3,23
	M	4381	100	2,28	108	2,47	4379	98	2,24	106	2,42
Diff. and sum		7666		1,07		0,94	7658		0,93		0,81
4	F	1063	25	2,35	23	2,45	1063	25	2,35	26	2,45
	M	2105	52	2,47	53	2,52	2105	52	2,47	53	2,52
Diff. and sum		3168		—0,12		—0,07	3168		—0,12		—0,07
Total	F	7892	242	3,06	245	3,10	7885	235	2,98	241	3,06
	M	9482	224	2,36	238	2,51	9473	215	2,27	228	2,41
Diff. and sum		17374		0,70		0,59	17358		0,71		0,65
Significance level, *P*, of the average differences				0,02		0,02			0,006		0,009
Significance level, *P*, of the differences of the totals				0,005		0,02			0,004		0,009

24—48, and 48—72 hours after irradiation. There was, however, not found any decided trend in the variation of the frequency of lethals with increasing length of time. The tentative assumption made to explain the difference in frequency of lethals after irradiation of impregnated females and of males could, hence, not be proved. In the tables we have, therefore, pooled the results from the different time intervals.

The cases in which the males were irradiated were, in their turn, subdivided into two different modes of experimentation, *viz.* mating the irradiated males to virgin females which in one group were, and in another group were not, previously irradiated. But there was not found any trend in the differences in lethal frequencies between these two ways of producing offspring of irradiated males, and the results from them are as well pooled in the table.

The comparisons to be made will, thus, only take into account the difference in the frequency of sex linked lethal mutations in the paternal chromosome when it is irradiated in impregnated females *vs.* when it is irradiated in males. We shall, in other words, confine ourselves to try to find out, from our new experiments, if this difference, as indicated in our previous article (BONNIER, LÜNING and ARNBERG, 1952), is a true one. Now, several of the experiments were made at different occasions but the two types, irradiation in impregnated females and in males, were always made more or less simultaneously. The best comparison seems, therefore, to be always to compare simultaneous experiments. Table 1 includes 4 different sets of experiments within each of which the irradiations of impregnated females and of males, respectively, were made more or less simultaneously. (Set nr. 1 includes the same experiments referred to as series Bf and Bq + Br in the cited article, BONNIER, LÜNING and ARNBERG, 1952.) In all of these experiments the dose was 960 r. The statistical evaluation of the comparisons (found at the bottom of the table) shows that the differences are significant, and this holds true whether we discard F_2 vials with no *M5* males and only few *y w sn* males according to method A or according to method B, and also irrespective of if we include semi lethals or not.

As said above, the two ways of irradiating the spermatozoa were, within each of the four sets, made more or less simultaneously. But they were, however, not irradiated under one and the same x-ray beam. Within two more sets of experiments (Table 2) the mode of experimentation was, therefore, as follows. Two to three days old *M5* males were

TABLE 2. *The production of recessive lethals in the M5 X-chromosome after irradiating spermatozoa with 3000 r. The M5 males were mated to y w sn females and the irradiation was given to impregnated females (F). The very same M5 males were, after their mating to the said y w sn females, themselves irradiated and mated to new virgin y w sn females (M). The experiments were performed at two different occasions (sets 5 and 6). Within each of the sets the males and the females were treated simultaneously by the same x-ray beam. In these experiments no semi lethals were found. The groups corresponding to A and B in Table 1 were identical.*

Set	Mode of irradiation	Number of tested chromosomes	Number of lethals	
			Abs.	%
5	F	370	31	8,38
	M	550	43	7,82
Diff. and sum		920		0,56
6	F	600	57	9,50
	M	463	27	5,83
Diff. and sum		1063		3,67
Total	F	970	88	9,07
	M	1013	70	6,91
Diff. and sum		1983		2,16
Significance level, *P*, of the average differences				0,08
Significance level, *P*, of the differences of the totals				0,08

mated to virgin *y w sn* females. After one day all the animals, males and females, were etherized and irradiated together under the same x-ray beam — in these sets with 3000 r. Immediately afterwards the males were separated from the females and mated to new untreated virgin *y w sn* females. From these matings eggs were collected during the first three days. The experiments in these sets were made on a smaller scale and the statistical significance of the differences between the percentages of lethals after the two modes of irradiation is, hence, not as strong as in the sets of Table 1. The difference is still very marked.

IV. DISCUSSION.

The figures from our experiments show decidedly that a certain x-ray dose produces a higher rate of lethals in the X-chromosomes of

spermatozoa if they are stored within females than if they are stored within males. Now, similar experiments have been made by earlier investigators (Table 3), and they have, in fact, got similar results. TIMOFÉEFF-RESSOVSKY (1937) found, after a dose of 3000 r, 9,76 per cent sex linked lethals when irradiating impregnated females, and 8,73 per cent sex linked lethals when irradiating males with the same dose. Obviously, however, he found the difference to be too small to be worth while mentioning. GULBEKIAN (1936, Table 3), on the other hand, got a very marked difference after the two modes of irradiation. He stresses this difference, pointing out that there may be two possible causes, (1) biological differences between males and females, and (2) irradiation of the plasma in the one, and non-irradiation of the plasma in the other case. His second explanation coincides with the tentative assumption made in our earlier publication (BONNIER, LÜNING and ARNBERG, 1952). However, if this explanation was correct it would follow that the same increase in the rate of sex linked lethals would be found after irradiating both males and females, the latter prior to insemination. As said above, we have not found such an increase. Moreover, the figures from TIMOFÉEFF-RESSOVSKY in Table 3 are pooled from two experiments, *viz.* (1) irradiation of both sexes, the females as virgins, when he got 8,42 per cent lethals, and (2) irradiation of males only, in which case he got 8,84 per cent lethals. We may thus conclude that our earlier assumption was correct insofar as a given dose of x-rays gives a higher rate of recessive sex linked lethals

TABLE 3. *Results of earlier investigations on the difference in rates of sex linked lethals after irradiation of spermatozoa from wild type males when the sperm is either irradiated in impregnated females (F) or in males (M).*

Author	Dose, r	Mode of irradiation	Number of tested chromosomes	Number of lethals		Significance level, *P*, of difference
				Abs.	%	
TIMOFÉEFF-RESSOVSKY (1937)	3000	F	891	87	9,76	
		M	3023	264	8,73	
Difference					1,03	0,35
GULBEKIAN (1936)	?	F	997	109	10,93	
		M	1885	121	6,42	
Difference					4,51	0,00008

in the male chromosomes if these are irradiated in impregnated females as compared with the effect of irradiation of males. This difference seems, however, not to be caused by any effect of the irradiation of the egg cytoplasm.

We do not know what the real cause of the difference is. One possibility is perhaps that spermatozoa are in a more sensible state with regard to their inclination to produce lethal mutations when stored in the somewhat more movable condition in the females than in the more rigid bundles within the testes. That the state of the cells may influence the rate of mutational processes has recently been shown by LÜNING (1952).

It may, in this connection, be mentioned that we still have results showing that the x-ray irradiation *per se* of females acts as enhancer of the mutational effect of x-ray irradiation of the male chromosomes. Such an effect does, however, not show on the rate of recessive lethals but on the production of a special kind of fractionals. In a coming publication this will be dealt with in detail.

SUMMARY.

(1) It is shown that x-ray irradiation of male X-chromosomes of *Drosophila melanogaster* produces a higher rate of recessive lethals if the irradiation is given to spermatozoa which are stored in impregnated females than if the irradiation is given to males.

(2) This effect is probably not caused by the irradiation *per se* of the females, but may perhaps be due to a difference in the state of the spermatozoa when being within the seminal receptacles of the females as compared with their state within the males.

Addendum at the proofs. — In a new series in which *M5* females were crossed to wild type (Canton S) males, inseminated females, virgin females, and males were simultaneously irradiated with 3000 r. The results from mating irradiated males to virgin females are, as before, practically identical whether the females were previously irradiated or not. Pooling these two types of matings the results were as follows. Corresponding to method A of Table 1: irradiation of inseminated females, 661 chromosomes tested, 8,77 per cent lethals; irradiation of males, 1583 chromosomes tested, 7,88 per cent lethals. The difference

is not significant in itself but after pooling with the results of Table 2 (in which the dose was 3000 r) the significance level, *P*, is 0,02.

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A MICROPLANIMETER FOR MEASUREMENT OF ENDONUCLEAR STRUCTURES

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THE determination of the volume of the nucleolar apparatus and of the nucleus is being used more and more as a measure of the speed of growth of cells and tissues. The background to this is the observation that there is a relationship between the size of certain parts of the nucleolar apparatus and the speed of the cytoplasmic protein synthesis.

For such measurements a rapid and reliable method for the determination of the linear dimension or optical cross sectional area of very small objects is necessary and the current methods offer difficulties of several different types. An ideal method should fulfil the following conditions: (1) It should work in a range of dimensions where the resolution of the microscope can be used to its very limit. (2) It must be possible to choose immediately the exact focal plane through the object where the measurement is to be made. One reason for this is that it is necessary to know that the largest cross sectional area of the particle to be measured is located in the histological section used and not in one of the adjacent ones. (3) It must be possible to make the measurements rapidly and also in an accumulating fashion, so as to get, within reasonable time, an average value of, for instance, the nucleolar size in a large cell material. (4) It must be possible to measure every accessible cell, because it very often happens in studies on the mechanism of growth that only a few cells in the same state of function are available.

Of the methods in use till now, those involving microprojection of the object and measurement in the projected image do not allow for the full use of the resolving power of the best high power microscope lenses. Photographic procedures do allow for such full resolving power. These methods are, however, extremely time-consuming and offer difficulties with regard to condition (2) above. Statistical methods, such as that developed by CHALKLEY, do not fulfil condition (4). The measurement of one or two diameters of a small object is subject to great errors, when compared with planimetric methods.

With the above mentioned requirements in mind, a microplanimeter, the construction of which is described below, has been designed. The basic principle is the following: A large thin lens deviates a narrow ray of light as does a prism. A weak lens will, however, influence the convergence of the beam very little. This prism-like effect is largest at the edge of the lens and decreases to zero at the centre, where the lens works as a plane parallel plate. This means that if a lens is moved in a plane perpendicular to a narrow beam of light, this beam will be deviated at an angle, the size of which will depend upon the distance between the lens centre and the point of intersection of the beam on the lens. If the beam gives an image of the object behind the lens, as indicated in Fig. 1, a movement of the lens will be followed by a movement of the image. The relation between the movement of a lens and the movement of the image is determined by the refractive power of the lens.

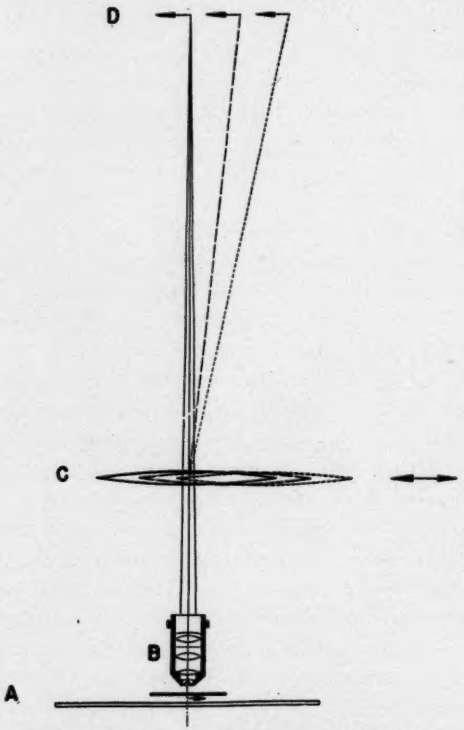

Fig. 1. A. Object. — B. Microscope objective. — C. Movable lens. — D. Image.

The microplanimeter (Fig. 2) consists of a microscope system of the highest class (B). A large lens with a diameter of ca. 10 cm and a refractive power within the range 0,2 to 2 dioptres (C) is placed closely behind the microscope objective. The refractive power chosen for this lens depends upon the range of dimensions of the objects to be measured. Since this lens does not influence the quality of the microscopic image the full resolution of the microscope system can be used. When the lens is moved in a plane perpendicular to the optical

axis of the system, the image, seen in the microscope eye-piece, moves in relation to a very fine hair-cross in the focal plane of the eye-piece. With the refractive powers indicated above, there is a linear relation between the movement of the lens and the movement of the image in the eye-piece over the whole distance from the lens centre to the lens periphery. When the lens is moved, the observer's impression is that the hair-cross is moved around the image of the object (H). When the

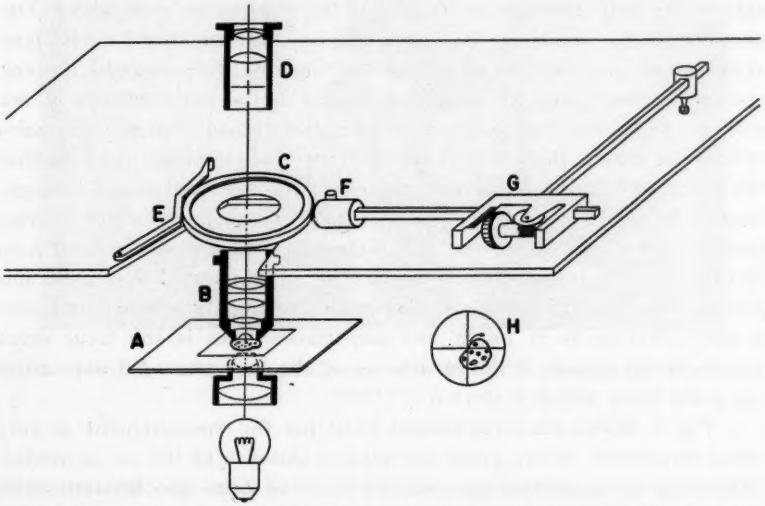

Fig. 2. Diagram of microplanimeter. — A. Object. — B. Objective. — C. Lens holder. — D. Eye-piece with fine hair-cross. — E. Attachment for centering. — F. Joint between lens holder and planimeter. — G. Planimeter. — H. Diagram of field of vision in the instrument at the measurement of a cell nucleus.

lens holder is connected with a planimeter system of conventional design and the hair-cross moved around, for example, a cell nucleus, the reading on the planimeter gives a relative measure of the optical cross section of the nucleus. The relation between the movement of the lens and the movement of the microscopic image depends upon the refractive power of the lens. By choosing a suitable lens it is possible to find an arrangement in which it is convenient to work in any dimensional range of the microscopic objects. Using a high power oil immersion lens which magnifies 100-times and an orthoscopic eye-piece with a magnification of 28-times it is convenient, in mammalian

material, to use a lens with a refracting power of 0,25 to 0,50 dioptres for nucleolar measurements and a refracting power of 0,5—1,0 dioptres for cell nuclei. It is of decisive importance that the measurement of an individual structure ends at exactly the same point where it was started. For this reason a special attachment marked E in the figure was incorporated in the apparatus. A simple manipulation of this attachment brings the lens back, with great accuracy, to a defined position, the most suitable position for this is that at which the optical axis of the lens coincides with that of the microscope's objective. The measurement is made in the following way: the moving lens is fixed at the given position, the object to be measured, for example, the cell nucleus in the figure (H) is moved by aid of the moving stage of the microscope so that one point on its periphery passes through the hair-cross. The lens is then moved till the hair-cross appears to go around the periphery of the nucleus and back to the starting point. Arrangement E is then used to bring the lens holder back to the exact starting position. The reading on the planimeter gives the cross sectional area of the cell nucleus in arbitrary units. The attachment E is of great importance for the measurement. The small irregularity in movement that it may produce is of much less importance than is the large error which would appear if the movement of the hair-cross did not end at the point from which it started.

Fig. 3 shows an arrangement built for the measurement of very small structures, where great mechanical stability of the set is needed. The optics of the microscope consist of a set of Zeiss' apochromates with a panchratic illumination apparatus and a high power orthoscopic eye-piece with an especially constructed fine hair-cross. The tube of the microscope is removed and the eye-piece held by a holder. The plane of the table top is just above the back focal plane of the microscope's objective. On this table a brass plate with a central hole is arranged. The lens holder slides on this plate. The lens holder is so built that lenses can easily be shifted. It is connected with a planimeter of conventional construction by a joint, worked with very high precision, arranged so that movements are possible in two directions but that the optical centre of the lens is always in the same location with regard to the arm of the planimeter.

If individual determinations are to be made, the planimeter must be read at the end of each measurement. The mechanism also has the advantageous feature, however, that a large number of measurements can be accumulated. It is possible, for example, to accumulate measure-

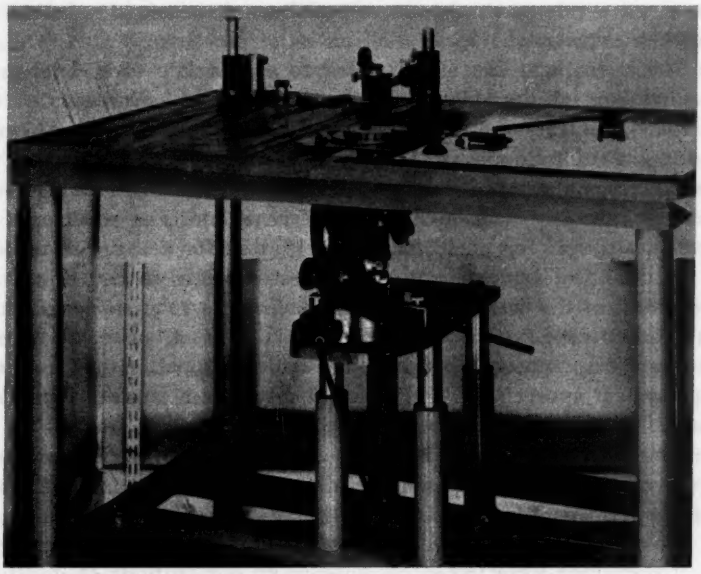

Fig. 3. Photograph of a microplanimeter, built especially for work on very small structures.

ments of 100 cells and so read the average cell area on the planimeter scale.

If one works at the highest magnification at the limit of resolution of the microscope, the boundary of the object to be measured does not appear absolutely sharp to the eye, even if ideal objects are used. This results from the fact that the work is done at a range of dimensions where the resolution of the microscope lens falls rapidly. It might be expected that the measurements of such structures would show great dispersion that resulted from this unsharpness. This dispersion is, however, not very great for the observer unknowingly picks out a certain level of light energy and follows that level around the object. The reproducibility is, however, much better if phase contrast optics are used even on stained preparations than if ordinary light optics are used. Not only is the definition of the boundary improved thereby, but a small increase in the resolution also results. The facts just mentioned will, of course, be a source of error in the measurement of very small

objects. As a rule, such objects will produce a measured value larger than their actual size.

The instrument can also be used to accumulate several series of measurements at the same time, for instance, measurements of the nucleolar-, nuclear- and cellular dimensions of the same cell. This can be done in the following way: 2 or 3 planimeter lens systems of the type described above are arranged above each other and suitable lenses chosen for the measurements of the different objects — weak ones for nucleoli, stronger for nuclei and still stronger for cell measurements. By the use of an arrangement of the type described in E of Fig. 2 the optical axis of the lenses not used can be brought to coincide with that of the microscope system. In this way as many as 3 lenses can be used simultaneously without disturbing the optical properties of the microscope system appreciably. This is especially true if a green light filter is used.

The calibration of the instrument was made by measurements of a square-net, ruled on a glass plate in a grating ruling engine. The side of the squares is 4 μ.

Tests of the planimeter. — The reproducibility and the size of the measuring-error were tested on a series of preparations of dog liver, fixed in formaline and stained with hematoxyline-eosine. Optics: Zeiss phase-contrast, oil immersion lens 90 $\times$, orthoscopic eye-piece 28 $\times$, green light filter, centre at 550 m. For nuclei the movable lens had a refractive power of 0,5 dioptres, for nucleoli 0,25 dioptres.

To get the measuring-error, ten nuclei (identified by numbers 1, 2 ... in the table) were measured after each other in numerical order. This series was then repeated 10 times and the mean and the standard deviation were calculated for each nucleus. The results were as follows:

Nucleus	Area in μ^2	Standard deviation in μ^2
1	38,8	0,28
2	35,9	0,36
3	35,9	0,31
4	36,1	0,39
5	40,0	0,39
6	38,4	0,42
7	33,7	0,42
8	36,2	0,36
9	38,4	0,42
10	40,7	0,39

The mean value of the standard deviation amounts to 0,4 μ^2 or about 1 %.

Different series with modified arrangements up to series of ten measurements around the same nucleus, repeated 25 times, all gave dispersions of the same size, and thus 3 % and 1,2 μ^2 represent the error of the individual measurement according to the conventional definition.

Optically it is to be expected that the width of the area of unsharpness around the nucleus would be of the order of magnitude 0,2 μ and thus the total area of the ring 4 μ^2. Within that area a reproducibility of 1 μ^2 is attained, explained by the fact that the eye picks out a certain intensity level around the nucleus.

Measurements on a large material of nuclei in the same preparation gave the mean 40,4 μ^2, with the standard deviation 0,98 μ^2. This shows that the physiological dispersion is considerably greater than dispersion due to measuring-errors.

Similar measurements were carried out on nucleoli of different sizes in a liver preparation from a dog with infectious hepatitis, where nucleoli of different orders of size can be found. The table below gives examples of measurements of individual nucleoli from this material. Working with these very small objects, the measurement is made so that the hair-cross of the planimeter is moved ten times around the object and the resulting reading divided by ten.

Nucleolar area in μ^2	Standard deviation in μ^2	Standard deviation in %
14,7	0,59	4
9,8	0,46	5
6,8	0,32	5
5,1	0,28	6
2,9	0,21	7
1,7	0,17	10
1,2	0,18	15

The table shows that, measuring smaller and smaller objects, the relative error of measurement tends to increase, but that the absolute error tends to decrease to a limiting value. This limiting value approximates the order of magnitude of the square of the smallest resolvable structure, defined according to Abbe $\left(\frac{0,550}{1,3}\right)^2 = 0,19\ \mu^2$.

It should be observed that because of the irregular shape of many nucleoli, especially the large ones, the measurement is more difficult than for rounded objects, which is one explanation to the fact that for large nucleoli the absolute error can be greater than for nuclei. In a normal liver section the mean of measurements of 100 arbitrarily

chosen nucleoli was 1,75 μ^2, with a standard deviation of 0,21 μ^2. This is below the range of dimensions where it is possible to determine the physiological standard deviation.

Summary. — A microplanimetric procedure is described, developed for the determination of optical cross sectional areas of microscopic objects. Special emphasis is laid upon the measurement of very small objects with high accuracy.

FURTHER STUDIES ON CHEMICALLY INDUCED REVERSIONS AT THE ADENINE LOCUS OF NEUROSPORA

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INTRODUCTION.

DURING the last five years the mutagenic effect of various chemicals has been studied in our laboratories by means of the *Neurospora* back-mutation test. A detailed account of the technique, by which the reverse mutation rate of a gene for adenine-dependence to adenine-independence is measured by a screening technique, has been published recently (JENSEN, KIRK, KÖLMARK, and WESTERGAARD, 1951). The validity of the technique has been criticized recently (GRIGG, 1952), and the criticism has been refuted (KÖLMARK and WESTERGAARD, 1952). In the above-mentioned publication (JENSEN *et al.*, 1951) were listed a number of chemicals, capable of increasing the reverse mutation rate of the adenine gene. It was pointed out that these substances, although unrelated in regard to their chemical structure, had the common property of being chemically very reactive compounds, producing free radicals or highly reactive ions. These positive results were discussed in relation to negative results obtained by chemically much more stable compounds such as urethane and certain phenols, which are known to be very effective in inducing chromosome breakage in plants and animals.

In the list of mutagenic chemicals submitted in the previous publication (JENSEN *et al.*, Table 9, *l. c.*) negative results were reported after treatment with ethyleneimine and ethylene oxide. These findings were surprising for various reasons: (1) Both compounds have three membered rings, and although not likely to react by a free radical mechanism, such three membered rings are known to be rather unstable, and open up easily with a concomitant release of energy. (2) The ring of ethyleneimine is likely to be produced during the complicated decomposition process of »mustard gas» which is a very mutagenic compound. (3) Ethyleneimine and ethyleneimine derivatives not only are very effective in breaking plant chromosomes (SJKVARNIKOV, 1948; LOVELESS and REVELL, 1949) but also induce lethals in *Drosophila*

(RAPOPORT, 1948; BIRD, 1952); these lethals are probably due both to chromosome mutations and to point mutations. Also ethylene oxide and a number of bis-epoxides have a very strong mutagenic effect on *Drosophila* lethals (RAPOPORT, 1948; BIRD, 1952) besides being very effective in inducing chromosome breakage (LOVELESS and REVELL, 1949; FAHMY, oral communication). These considerations indicated the necessity of a re-examination of the mutagenic effect of these chemicals on *Neurospora*, and the present paper presents the result of a number of new experiments.

MATERIAL AND METHODS.

The method for screening reverse mutations from adenine-dependence to adenine-independence is the same as that previously described (JENSEN *et al.*, 1951; KÖLMARK and WESTERGAARD, 1949) except that a more extreme colonial mutant has been used here. The original experiments were carried out with a colonial mutant (70.007) obtained by mustard gas treatment by Dr. G. W. BEADLE. This colonial mutant was crossed to the purple, adenineless strain from which cross the double mutant colonial, adenineless was isolated. This double mutant was designated W. 40. In mutation experiments with this strain a new colonial type appeared. It formed even smaller colonies, and proved more convenient for plating and counting. This strain was adenine-dependent, and designated W. 40, »circularis». From this strain a new extreme colonial type, likewise adenine-dependent, originated in the course of a mutation experiment. This strain forms extremely small, very distinct colonies, and has proved superior to the former colonial mutants; it is now used in all experiments, and has been designated W. 40, »distinctus». Even after a week of incubation the size of the colonies is only 3—5 millimeters in diameter, and approximately 100 colonies can easily be counted on a 8×8 centimeter Petri dish. Unfortunately, the genetic relationships of these colonial mutants has not yet been worked out, but they are all very stable, reverse mutations to more spreading growth types being very rarely encountered.

The extreme colonial mutant has the only disadvantage that conidiation is often very scanty. This difficulty has been satisfactorily overcome by using a medium which stimulates conidiation. This medium contains, besides the standard ingredients of the FRIES »complete» medium (BEADLE and TATUM, 1945), an extract of *Neurospora* mycelium. It is prepared as follows: To 1000 ml of FRIES »complete» medium are added 50 gms of fresh, wild type mycelium. The mycelium

is harvested from a culture, grown for 8 days at 25° C in a 5 liter flask in liquid »complete» medium with aeration. The liquid is removed by pressing the mycelium on filter paper, the mycelium is cut up in a WARING blendor, and 50 gms of the chopped mycelium are added to 1000 ml FRIES »complete» medium. The medium is solidified through the addition of 1,5 per cent agar. It has been found that stimulation of conidiation by this medium is restricted to the adenineless strain, whereas it has no effect on other strains.

EXPERIMENTAL RESULTS.

Ethyleneimine $(CH_2)_2NH$. — In previous, negative experiments, ethyleneimine was first applied by dissolving it in dry ether, which was then added to a suspension of conidia in sterile water. This method had proved satisfactory for treatment with diazomethane (JENSEN, KÖLMARK, and WESTERGAARD, 1949). In other experiments pure, liquid ethyleneimine was added directly to the spore suspension, but both treatments gave completely negative results (JENSEN *et al.*, 1951). In the present experiment ethyleneimine was added to the spore suspension in the gas state by the following procedure: Ethyleneimine is prepared from β-bromoethylamine-hydrobromide following the method of GABRIEL and STETZNER (1895). 20 gms of β-bromoethylamine-hydrobromide are mixed with 50 ml of 33 per cent KOH. First an oil $(BrCH_2—CH_2—NH_2)_n$ is formed; it dissolves with liberation of HBr. The solution is then boiled for a few minutes using a reflux condenser, and $H_2C—CH_2$ is formed. The flask is cooled to 50° C, and placed on
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TABLE 1. *Ethyleneimine* $H_2C—CH_2$ (NH) *0,05 M.*

Minutes treated	Per cent survived	Mutations	Mutations 10^6 initial	Mutations 10^6 survived
Control *	100	8	0,13	0,13
8	100	290	4,6	4,6
18	100	605	9,8	9,8
28	76	974	15,6	20,5
38	28	969	15.5	55.4
50	1	71	1,1	134,0

* 63×10^6 conidia.

These results encouraged a further investigation into the mutagenic effect of ethyleneimine derivatives, some of which have proved to have a very strong effect in inducing *Drosophila* lethals (BIRD, 1952), and some of which also have been shown to have very interesting clinical effects on certain types of tumours (HENDRY, HOMER, ROSE, and WALPOLE, 1951 b). For preliminary investigations 2 : 4 : 6-tri (ethyleneimino)—1 : 3 : 5-triazine (T. E. M.), kindly provided by the Chester Beatty Institute, London, was selected. T. E. M. has by far the strongest mutagenic effect on *Drosophila* (BIRD, 1952), and the effect on plant chromosomes is very similar to that obtained by X-ray treatment (REVELL, 1952; unpublished thesis); besides, it has been found to have a very interesting clinical effect on HODGKIN's disease (for references, see BICHEL, 1951). Preliminary experiments have shown that T. E. M. is very toxic to *Neurospora* conidia, and no mutagenic effect on the adenine locus has so far been demonstrated. However, the experiments cannot be considered conclusive and will be continued.

Ethylene oxide and bis-epoxides. — In some preliminary experiments on *Neurospora*, ethylene oxide was applied in the same way as diazomethane, *i. e.*, by dissolving it in dry ether, and subsequent addition of this solution to a suspension of conidia. The results were completely negative. Encouraged, however, by the results obtained with ethyleneimine, a similar technique was employed for the application of ethylene oxide. Ethylene oxide from a steel flask containing compressed $(CH_2)_2O$ in the gas state was received into distilled water, and the concentration determined by titration. During titration an excess of KCl is added, after which the following reaction takes place:

$$\begin{matrix} H_2C \\ | \\ H_2C \end{matrix}\!\!>\!O + KCl + H_2O \rightarrow \begin{matrix} CH_2Cl \\ | \\ CH_2OH \end{matrix} + KOH$$

TABLE 2. *Ethylene oxide* $H_2C—CH_2$ (O), *0,025 M.*

Minutes treated	Per cent survived	Mutations	Mutations 10^8 initial	Mutations 10^8 survived
Control *	100	8	0,13	0,13
7,5	73	273	4,5	6,2
15	63	1023	16,9	27,0
25	10	679	11,2	107,8
35	0	0	0	0

* 60×10^6 conidia.

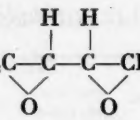
The hydroxyl ion is then titrated with 0,1 N HCl, using methyl red as indicator. The solution is heated to *ca.* 80° C during titration.

Again this method of treatment gave very good results. Table 2 shows an experiment in which conidia have been treated with 0,025 M ethylene oxide, the time of treatment varying from 7 minutes to 35 minutes.

The mutagenic effect of ethylene oxide was first demonstrated on *Drosophila* ClB stocks by RAPOPORT (1948). The results were confirmed by BIRD (1952), who also undertook an investigation of the mutagenic effect of various bis-epoxides (BIRD, 1952; BIRD and FAHMY, oral communication) some of which show a remarkably strong effect on *Drosophila.* Besides, the effect of the compounds on plant chromosomes has been studied by REVELL (*l. c.*). Some epoxides were kindly put at our disposal by the Chester Beatty Institute; the two compounds provided were 1 : 2,3 : 4-diepoxybutane and di-(2 : 3-epoxypropyl)-ether (*cf.* HENDRY, HOMER, ROSE, and WALPOLE, 1951 a).

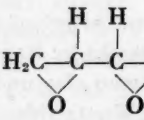
Tables 3 and 4 show two mutation experiments with diepoxybutane. In Table 3 the number of mutations as a function of concentration during a 25 minute treatment are recorded, and Table 4 shows the mutation yield as a function of time using optimal concentration (*ca.* 0,2 M diepoxybutane).

Diepoxybutane has a very strong mutagenic effect. As a matter of fact, in the first experiments it was impossible to count the number of mutations on the minimal plates, because the colonies were too numerous, and it became necessary to dilute the conidia suspension by a factor of 10 in order to be able to count the colonies on minimal. In Table 4 it is seen that the optimal period of treatment (40 minutes) yields almost 16.000 colonies against 5 in the control (the »background«), *i. e.*, the number of mutations is increased from less than 3 in 10^8 conidia to almost 1 in 10^4 conidia. This is about 5 times as

TABLE 3. *1 : 2,3 : 4-Diepoxybutane*  *· 25 minutes treatment.*

Concentration	Per cent survived	Mutations	Mutations 10^6 initial	Mutations 10^6 survived
Control *	100	62	0,29	0,29
0,01 M	100	46	0,22	0,22
0,05 »	96	360	1,7	1,8
0,1 »	53	1400	6,6	12,4
0,15 »	29	3800	17,8	62,3
0,2 »	25	6640	31,2	128,0
0,25 »	6	6940	32,6	598,0
0,3 »	2	3760	17,7	1044,0
0,4 »	0,003	2	0,009	∞

* 213×10^6 conidia.

TABLE 4. *1 : 2,3 : 4-Diepoxybutane*  *· 0,2 M.*

Minutes treated	Per cent survived	Mutations	Mutations 10^6 initial	Mutations 10^6 survived
Control *	100	5	0,03	0,03
5	100	26	0,14	0,14
10	100	380	2,1	2,1
20	100	1335	7,3	7,3
30	92	6120	33,4	36,4
35	76	10120	50,4	72,8
40	56	15580	85,2	151,2
45	39	15030	82,0	211,2
50	14	8640	42,2	346,0

* 183×10^6 conidia.

good an effect as that obtained by ethylene oxide, and 20 times better than the effect obtained by mustards, ultraviolet light, and other so-called strong mutagens (see later). However, not all bis-epoxides induce a similar strong enhancement of the mutation rate at the adenine locus. This is clearly shown in Table 5, which gives the effect of treatment with diepoxypropylether. Although the effect is significant, it is of a quite different order of magnitude than that obtained with diepoxybutane, and this compound is far less mutagenic than, *e. g.*, ultraviolet light or mustard gas.

TABLE 5.

Diepoxypropylether $H_2C(O)CH-CH_2-O-CH_2-CH(O)CH_2$ *0,01 M.*

Minutes treated	Per cent survived	Mutations	Mutations 10^6 initial	Mutations 10^6 survived
Control *	100	4	0,05	0,05
5	100	12	0,15	0,15
10	100	15	0,19	0,19
20	72	35	0,44	0,61
30	68	55	0,69	1,0
40	53	56	0,70	1,3
80	5	8	0,1	2,4

* 80×10^6 conidia.

Both our diepoxides contained the two isomers, and the two racemic forms have not yet been tried separately, although such experiments are in preparation. The technique for preparing the two isomers of diepoxybutane has been described by BEECH (1951). It should be added that diepoxybutane must be chemically very pure to give a good yield of mutations (for determining its purity, see ROSS, 1950). The samples provided to us by Dr. ROSS were approximately 96 per cent pure. We then tried to make the compounds ourselves by the method described by EVERETT and KON (1950), using butadiene as the starting material (for the preparation of butadiene, see KADESCH, 1946). However, our own samples proved more toxic, and gave a much lower mutation yield than the sample obtained from the Chester Beatty Institute, and we are greatly indebted to Dr. ROSS for providing us with additional material.

The interesting results obtained by diepoxybutane treatment raise a number of questions. The first one is, of course, whether all the colonies which appear on minimal medium are true back-mutations, or whether they arise through a different mechanism. Phenotypically they are identical with colonies induced by other mutagens, and they keep on growing on minimal medium after a number of transfers. So far, all reversions at the adenine locus behave genetically as true back-mutations, giving all-wild-type ascospores when crossed to wild type (JENSEN *et al.*, 1951; HOROWITZ, oral communication). Ten of the diepoxybutane induced colonies from minimal medium have been crossed to wild type by the usual technique, and they all behaved as true back-mutations, similar to the colonies obtained by other mutagens. Thus there is no indication at the present that the diepoxybutane induced colonies are different from those obtained with other mutagens.

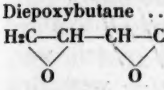
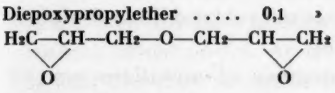
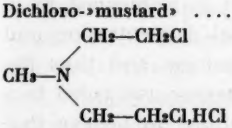
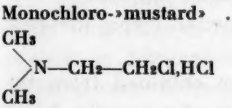
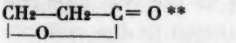
The second question is whether diepoxybutane might have a specific effect on the reverse mutation rate of the adenine locus, as might well be the case since the treatment increases the back-mutation rate of this particular gene (which can by no means be considered an unstable gene) by a factor of several thousand. If the compound had the same effect on all loci, one might almost expect it to produce mutations at any particular locus tested, an effect which a priori would seem rather unlikely. Two lines of experimental approach to this problem are obviously open. The first one is to study the effect of diepoxybutane on the reverse mutation rate of other genes; the second would be to study its effect upon forward mutation rates. The first method has already been used, and preliminary results are reported in a publication by KÖLMARK (1953). The ability of diepoxybutane to induce nutritional (»biochemical») mutations in *Neurospora* is now being investigated, but the experiments have not yet been concluded. It should be noted that diepoxybutane has been employed in the production of higher yielding penicillin-producing strains of *Penicillium chrysogenum* (HENDRY *et al.*, 1951 a). The experiments were only carried out on a small scale, and the results proved disappointing; it was concluded that the chemical was inferior to ultraviolet treatment in increasing the yield of the antibiotic.

Our results with epoxides and bis-epoxides should be considered as being only preliminary. However, they already strongly suggest that these compounds may be very useful for studying some of the problems related to the mutation mechanism, such as, *e. g.*, the relationship between chemical structure and mutagenic effect of closely related compounds (*cf.* the difference between the mutagenic effect of diepoxybutane and of diepoxypropylether). A very great number of diepoxides and polyepoxides have been synthesised (HENDRY *et al.*, 1951 a), and are thus available for study. Another important aspect of the biological effect of these chemicals is their capacity of promoting and inhibiting certain types of tumours (HENDRY *et al.*, *l. c.*). (This approach has, of course, been clearly emphasized by the team at the Chester Beatty Institute.) Thus the epoxides may also be useful in exploring the much discussed problem of the relationship between mutations and carcinogenesis.

DISCUSSION.

Table 6 summarizes our present informations about chemical and physical mutagens able to enhance the back-mutation rate at the adenine

TABLE 6.

Mutagen	Concentration	Time	Per cent survived	Mutations 10^6 initial	Mutations 10^6 survived
Diepoxybutane 	0,2 M	40 min.	50	85,2	151,2
Ethylene oxide 	0,05 »	10 »	12	18,3	144,0
Diepoxypropylether 	0,1 »	20 »	65	0,7	1,0
Ethyleneimine 	0,05 »	30 »	75	15,6	20,5
Dichloro-»mustard« 	0,0025 »	25 »	60	3,4	5,8
Monochloro-»mustard« 	0,005 »	60 »	80	1,7	2,1
Hydrogen peroxide + Formalin H_2O_2+HCHO	0,06 » + 0,03 »	30 »	20	4,3	46,0
Hydrogen peroxide H_2O_2	0,2 »	45 »	10	0,4	4,2
Formalin, HCHO	0,01 »	3 hours	80	0,3	0,4
Diazomethane CH_2N_2	0,3 »	40 min.	20	0,6	3,4
Ultraviolet light	—	—	53	3,9	7,4
X-rays, 25000 r	—	—	65	3,2	5,0
tert-butyl-hydroperoxide $(CH_3)_3C—O—O—H$ *	0,09 M	30 min.	50	1,5	3,0
β-propiolactone 	0,03 p. c.	60 »	?	3,8	?

* Data from DICKEY *et al.*, 1949.

** Data from SMITH and SRB, 1951.

locus of *Neurospora*. The table also includes two chemicals which have been studied by other authors. We do not claim any priority for the discovery of the mutagenic effect of any of these compounds. As a matter of fact, all of them — except the mustards which were discovered by AUERBACH and ROBSON (1946), and the peroxides which were discovered by WYSS, CLARK, HAAS, and STONE (1948) — were first used by RAPOPORT, employing the *Drosophila* ClB technique. Of the mutagenic chemicals discovered by RAPOPORT, we have so far obtained negative results with ketene only (*cf.* RAPOPORT, 1947 a), but the experiments were not satisfactory. We have not yet tried diethylsulphate and dimethylsulphate (RAPOPORT, 1947 b).

Table 6 also shows the maximal number of mutations per 10^6 treated conidia, under optimal conditions. Most of these figures have been confirmed by a number of experiments. On the whole we find that the number of mutations obtained per 10^6 conidia remains rather constant from one experiment to another, but it is influenced by a number of external factors, especially the conidial concentration and the age of the conidia used. The survival percentage, and thus the number of mutations per survived conidia, is, however, subjected to a much greater degree of fluctuation. We are inclined to believe that these fluctuations are at least partly due to experimental errors involved in spore dilution and pipetting.

We believe that valuable information can be obtained from the figures of this table, but unfortunately most of it is of a negative kind. For one thing, the figures clearly show that expressions like »strong» and »weak» mutagens are quite meaningless, at least if they are used in the sense of bearing any relationship to the mutation mechanism. The fact that treatment for 40 minutes with 0,2 M diepoxybutane (which represents optimal condition for this chemical) produced 20 times more mutations than a 0,0025 M concentration of a dichloro-mustard, applied for 25 minutes, may be of great practical interest to those who, for practical or theoretical purposes, want to produce large numbers of mutations, but it only tends to show that the efficiency of a given mutagen depends upon a great number of factors about which hardly anything is known at present. The mutagenic effect of a chemical depends on: (1) Its penetration across the cell wall (in plants) and the cell membrane. (2) Its diffusion in the cytoplasm of the cell. (3) The rate by which the chemical is destroyed or inactivated in the cell (its interaction with antimutagens or other factors). (4) Its ability to penetrate the nuclear membrane (*cf.* KIHLMAN, 1951). (5) Its reactivity

with the genetic material in the cell nucleus. (Most authors seem to take it for granted that chemical mutagens must reach the chromatin material and react directly with the DNA of the chromosomes, although this has not been proven in a single case, and may not be necessary at all.) (6) Last, but not least, the mutagenic effect has to be considered in relation to the toxic effect of the chemical, and it is very difficult to obtain a genetic system which allows the study of these two processes independently of one another.

We believe that the back-mutation screening system possesses a sufficient quantitative accuracy to furnish the answers to some of the questions stated above. At least two lines of approach seem feasible. The first one may be called the physico-chemical approach. It should be possible to study at least some of the questions related to problems of penetration, diffusion, and destruction of the mutagens in the cells by rather simple temperature experiments. As to the important problem concerning the penetration of the mutagen into the cell nucleus, it would seem tempting to approach this by using isotope-marked mutagens, though we fully realize the technical obstacles lying in the path of such experiments.

The second line of approach would be an extention of the methodology to studies of reverse mutations in other loci, and to quantitative studies on forward-mutations. The fact that diepoxybutane produces 20 times more mutations than X-rays, ultraviolet light or »mustard-gas» may appear in a wholly different light if it would be found that the picture was reversed for other genes in the same organism. As will appear from the publication by KÖLMARK (1953) such a situation has actually already been found in *Neurospora*, a discovery, which we believe opens up quite new problems concerning the dynamics of the mutation mechanism.

Table 5 includes only such chemicals which have shown a positive mutagenic effect on the adenine locus. However, these data should also be discussed in relation to our negative results with a number of other chemicals, and especially such compounds which have been shown to induce various types of mutations in other organisms. However, the validity and significance of negative experiments are critical points in mutation work (*cf.* our own previous reports concerning the inefficiency of ethyleneimine and ethylene oxide).

Anyone of the factors discussed above may, of course, also prevent a potential mutagen from producing any mutation at all: If the chemical is unable to penetrate the cell membrane, if it is destroyed or inactivated

too rapidly in the cytoplasm, if it is unable to penetrate the nuclear membrane or if it is so toxic that the killing effect masks the mutagenic effect, *etc.*, negative results may be obtained. This line of argumentation may well force us into accepting the hypothesis that all chemicals are potential mutagens, a proposition which may be quite true, but which, for the present at least, has little value as a fruitful working hypothesis.

In the work involving back-mutations in *Neurospora* we have been impressed by the pattern relating the mutagens to the mutagenic effect produced. All those chemicals, which have shown a positive mutagenic effect have, although quite unrelated as to chemical structure, some physico-chemical characteristics in common, *i. e.*, they are all chemically very reactive compounds, which, by producing free radicals (*e. g.* the peroxides or diazomethane), reactive ions (*e. g.* the mustards), or by possessing unstable three membered rings (*e. g.* the epoxides), are able to release free energy, and it is this burst of free energy which may be ultimately responsible for the mutagenic effect. The chemicals which have failed to induce mutations by the present test, such as phenols, urethane, manganous chloride, and others, have, on the other hand, quite different physico-chemical properties, being much more stable compounds. When these observations are considered in relation to the genetic and cytological tests by which the mutagenic effect of these compounds have been tested, it seems possible to form at least a working hypothesis for their mutagenic activity.

As pointed out in previous publications, the *Neurospora* back-mutation test may be considered a more specific test for gene mutations than the tests by which the mutagenic effect of urethane and of the phenols have been demonstrated in other organisms. It is known that they (as well as many other chemicals; for references, see KIHLMAN, 1952) are able to induce chromosome breakage and thus chromosome mutations. It is also known that phenol (HADORN, ROSIN, and BERTANI, 1949) and urethane (VOGT, 1950) are able to induce lethals in *Drosophila*. It is known from cytological inspection of the salivary chromosomes of *Drosophila* lethals that they may comprise both chromosome mutations and (by negative evidence, *e. g.*, from the absence of visible changes in the chromosomes) point mutations. Hence, it is quite likely that the urethane-induced lethals in *Drosophila* are all due to chromosome mutations.

Thus if our negative results with the »stable» compounds are accepted on their face value, and if one makes the hypothesis that these substances induce chromosome mutations only (a hypothesis open to

experimental verification), it seems possible to outline a scheme which may help to explain some of the contradictory results obtained during the very extensive work done on chemical mutagenesis during the last five years. Such a scheme would include the following considerations:

(1) A clear distinction between mutagens with a direct effect and those which act indirectly, by destroying or inactivating antimutagens. A very clear-cut example of this type of interaction is encountered in the hydrogen peroxide—catalase—potassium cyanide system originally discovered in bacteria (WYSS, CLARK, HAAS, and STONE, 1948) and confirmed by us in *Neurospora* (JENSEN *et al.*, 1951, Tables 4—5). Hydrogen peroxide is able to induce gene mutations probably through a free radical mechanism (JENSEN *et al.*, *l. c.*). The mutagenic effect stops, however, when hydrogen peroxide is destroyed by catalase, and it has been found that in cells with a high catalase activity, hydrogen peroxide is unable to increase the mutation rate (WYSS *et al.*, *l. c.*). Hence, catalase is a natural antimutagen. If, however, such cells are treated with potassium cyanide, which inactivates the enzyme, the mutation rate is increased (WAGNER, HADDOX, FUERST, and STONE, 1950; JENSEN, *et al.*, *l. c.*). However, potassium cyanide is not a mutagen in the same sense as hydrogen peroxide, but works by indirectly stabilizing the concentration of a mutagen of the first order. Similar antimutagens have been discovered against X-rays (HOLLAENDER, BAKER, and ANDERSON, 1951).

(2) A realization of the fact that the heterogeneity of the results obtained with mutagenic agents reflects to a certain degree the heterogeneity of the biological mutation concept. The concept »mutation» today covers many different things in biology. From the very beginning it comprised point mutations as well as chromosome mutations of various kinds. It has been extended by many authors to include also a number of cytoplasmic changes (»cytoplasmic mutations», »plastid mutations», *etc.*), changes which are not normally transmitted to the offspring through the sexual cycle. It now includes also a number of phenotypical changes in bacteria, about which it is generally not known whether they are cytoplasmic or nuclear in origin.

(3) It is not possible to form a general theory for chemical or physical mutagenesis, but the different categories of mutations must be discussed separately. Some chemicals induce point mutations, and transfer of free energy seems to play an important rôle in this process. Probably this type of mutagen is able to induce chromosome breakage as well. Other chemicals may induce chromosome breakage, but not

gene mutations, and the mechanism of chromosome breakage will have to be worked out separately for different chemicals. Other chemicals may induce »cytoplasmic mutations», the most thoroughly analysed system of this kind being that of the acriflavine induced small colony mutant in yeast, studied by EPHRUSSI and his co-workers (see EPHRUSSI and HOTTINGUER, 1951).

Obviously the usefulness of the above scheme is restricted to such organisms in which the genetical and cytological conditions for discriminating between the different categories of mutations are fulfilled. For the present at least, this is unfortunately not possible in the majority of the mutation work carried out with bacteria, and it is thus not possible to correlate a number of the very interesting observations on spontaneous and induced mutations in bacteria with the mutation pattern in organisms with a cytologically and genetically well established sexual system.

SUMMARY.

Back-mutations in an adenine requiring strain of *Neurospora crassa* have been induced by ethyleneimine, ethylene oxide, and two bis-epoxides. One of these, 1 : 2,3 : 4-diepoxybutane, produced more back-mutations than any other previously used mutagen. The results were 20—30 times better than those obtained by treatment with ultraviolet light, X-rays, and »mustard-gas».

The results are discussed in relation to previous results with other mutagens, and a list of the mutagens which are able to induce back-mutations in the adenineless strain is given. The data are discussed in relation to the theory of chemical mutagenesis.

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CYTOTAXONOMICAL REMARKS ON THE GENTIANACEAE

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I. INTRODUCTION.

IT is only during the last couple of years that the family Gentianaceae has attracted the attention of cytologists. But in this short time it has been found to contain a wealth of problems concerning almost everything of interest to cytotaxonomists. The family is considered a very old one, and its approximately 800 species have a world-wide distribution, from the Arctic to tropical climates as well as from the highest mountains to the lowlands and sea shores. Gentianaceae has been taken by a number of classical and modern taxonomists (*cf.* GILG, 1895; GUNDERSEN, 1950; FERNALD, 1950; LAWRENCE, 1951) in a broad sense, including also *Menyanthes*, *Nymphoides*, and related genera, while others have regarded it as more convenient to take it in its strict sense, excluding the family Menyanthaceae on basis of its morphology, anatomy (*cf.* LINDSEY, 1938), and other characteristics of taxonomical significance (*cf.* BRITTON and BROWN, 1897; WETTSTEIN, 1935; HYLANDER, 1941, 1945; JONES, 1950). The present author concurs with the latter opinion, thus excluding Menyanthaceae from the present study.

The family Gentianaceae in its strict sense has been arranged into several tribes with different genera and sections based on the morphology and anatomy of the species (*cf.* GILG, 1895; KUSNEZOW, 1895; WETTSTEIN, 1896). Some of the species of these subgroups have been found to include several geographical races taxonomically named as subspecies, varieties, or forms, but the family seems to be amazingly free from collective species, thus indicating a rather high age and firm stabilization of its species groups.

Besides a few remarks about its chromosome numbers by STOLT (1921), SCHEERER (1939), WOYCICKI (1932, 1933, 1937), SAKAI (1934, 1940), and SOKOLOVSKAJA and STRELKOVA (1938), it was not until RORK (1946, 1949) published her thesis work on a large number of species of Gentianaceae that the family began to be known cytologically. At about the same time, FAVARGER (1949, 1952) and SKALINSKA (1950, 1952) commenced to investigate the Gentians of the Alps and the Tatra

mountains, respectively. In the chromosome list by TISCHLER (1950) we also find some unpublished numbers given by Mrs. MATTICK-EHRENSBERGER, and KNABEN (1950) reports the numbers for two species from Norway. Thus, by now chromosome numbers are known for more than fifty different species of Gentianaceae.

The present author has been able to determine the chromosome numbers for all the seven species of Gentianaceae known to occur in Iceland. The material was collected in 1946—1950, and root-tips were fixed on the spot in the modification of Navashin's fluid given by MÜNTZING (1932). The cold climate of Iceland seems as a whole to be very suitable for fixations in nature and the results obtained with the present material were perfectly satisfactory, contrary to the experience of other authors who seem to have had considerable difficulties in getting good fixations.

All the Icelandic representatives of the family Gentianaceae belong to the indigenous element of the flora as defined by LÖVE and LÖVE (1951) and LÖVE (1953). They have all survived at least the last glaciation in Iceland.

II. CYTOLOGICAL RESULTS.

1. GENUS GENTIANA L.

A. SECTION *CYCLOSTIGMA* GRISEB.

The only species representing this genus in Iceland is *G. nivalis* L., which according to HULTÉN (1950) has an amphiatlantic, arctic-montane area of distribution although it also is present in some southern mountains. Cytologically, this species was first determined from the Alps by FAVARGER (1949) and later on from the Norwegian mountains by KNABEN (1950). Both found the chromosome number to be $2n = 14$, and the same number was counted in the Icelandic material.

2. GENUS GENTIANELLA MOENCH.

A. SECTION *ENDOTRICHA* FROEL.

G. Amarella (L.) H. SM. — This species is distributed over a wide area from western Europe into central Siberia, according to HULTÉN (1950). In Iceland it is represented by the arctic-montane race, ssp. *lingulata* (AG.) LÖVE et LÖVE. The chromosome number of the Icelandic material was found to be $2n = 36$, which is in good agreement with the numbers for other species of the same section, as counted by FAVARGER (1949, 1952) and SKALINSKA (1952).

G. campestris (L.) H. SM. — This is a European boreal-montane species with separate northern and southern areas (*cf.* HULTÉN, 1950). Taxonomically, it is a very variable type including several geographical races. In Iceland it is represented by the arctic-montane race, ssp. *islandica* (MURB.) LÖVE et LÖVE. In material of the ssp. *campestris* from the Alps, FAVARGER (1949) counted $2n = 36$ chromosomes, and the same number was determined for the Icelandic specimens.

B. SECTION *ARCTOPHILA* GRISEB.

G. aurea (L.) H. SM. — A representative of the amphiatlantic-arctic area of distribution (HULTÉN, 1950), this species has been found to have $2n = 36$ chromosomes in Iceland. The same number was reported by RORK (1946, 1949) for the American species *Gentianella quinquefolia* (L.) D. LÖVE, *comb. nova* (based on *Gentiana quinquefolia* LINNAEUS, 1753), belonging to the same section.

C. SECTION *COMASTOMA* WETTST.

G. tenella (ROTTB.) H. SM. — This small biennial Gentian, spread over a vast circumpolar, arctic-montane area (*cf.* HULTÉN, 1950), has the lowest chromosome number hitherto known within the family, or $2n = 10$, as demonstrated from the Alps by FAVARGER (1949), from the Norwegian mountains by KNABEN (1950), and by the present author on her Icelandic material.

D. SECTION *CROSSOPETALUM* FROEL.

G. detonsa (ROTTB.) G. DON. — Like the last-mentioned species, this type was originally described from Iceland, but later it was found to be fairly common in arctic Russia (*cf.* HULTÉN, 1948) and in northern and westernmost Scandinavia (*cf.* LID, 1944; HULTÉN, 1950), as well as in some localities in Greenland (M. P. PORSILD, 1935; BÖCHER, 1938). According to HULTÉN (1948), this species in its strict sense should also be represented at the arctic shore of the Mackenzie district and Alaska, but this view is criticized by A. E. PORSILD (1951), who regards the last-mentioned type as a clearly separate species. The chromosome number of the Icelandic material was found to be $2n = 44$. The same number was reported by FAVARGER (1949) for *G. ciliata* (L.) of the same section.

3. GENUS LOMATOGONIUM A. BR.

L. rotatum (L.) A. BR. — This is a circumpolar, arctic-montane species with a continuous distribution area in northern Siberia (*cf.* HULTÉN, 1950), although it is known from one locality only on the Kola

Peninsula and from arctic Russia, but not from Scandinavia. The chromosome number of the Icelandic plants was found to be $2n = 10$.

III. DISCUSSION.

In order to facilitate an interpretation of the present and previous cytological results for the taxonomical understanding of the family Gentianaceae in its strict sense, the chromosome numbers now known for its different species have been brought together in Table 1. This table also includes some remarks on the morphology of the chromosomes.

The separation of the family Menyanthaceae from Gentianaceae has been made on basis of morphological and anatomical differences. Only in a few cases can cytological distinctions be used as additional characters for separating families, and in the case of these two families the variations in the morphology and the basic number of chromosomes make these results of a little systematic value for their separation.

Quite different is the division into genera and their subgroups, as these must be regarded as a much more recent result of evolution than is the family level. It is understood that each genus and section in its strict sense should include only species evolved from the same original group, and here both morphological and numerical investigations on the chromosomes of different species can make fully reliable additional evidences for the subdivisions from an evolutionary standpoint.

The wide range of basic chromosome numbers in Gentianaceae, $x = 5, 6, 7, 9, 11, 13$, had already attracted the attention of FAVARGER (1949) and SKALINSKA (1952). Both these authors tried to explain the higher numbers $x = 11$ and 13 in different ways. FAVARGER (*l. c.*), who had not found the number $x = 6$ within the family, assumed the original number of the family to be $x = 5$, and that the basic number $x = 11$ had been formed through polyploidy followed by fragmentation. The number $x = 13$ should in the same way have originated from $x = 7$ by polyploidization and fusion. Hence, by combination of $x = 7 + 11$ he reaches $2n = 36$, and by $9 + 13$ to $2n = 44$.

SKALINSKA (*l. c.*), however, found *G. frigida* to have $2n = 24$, and thus, a basic number of $x = 6$. Therefore, her explanation, that we have the basic numbers $x = 5$, 6 and 7, and that $x = 11$ and 13 have arisen by simple combination and alloploidy from $x = 5 + 6 = 11$, and $x = 6 + 7 = 13$ is the most plausible one. The present author agrees with this opinion.

The division of the family Gentianaceae into genera and sections is not yet agreed upon by all taxonomists studying this group. The system used above is that by SMITH in HYLANDER (1945) as regards the divisions into genera of the Icelandic species, and that by KUSNEZOW (1895, 1896—1904) and WETTSTEIN (1896) concerning the division into sections. From morphological as well as cytological points of view, this division is, however, not satisfactory as these sections still include groups of considerable diversity.

There is no doubt that all the sections here referred to as subgroups of the genus *Gentianella* are morphologically very different from all the sections of the genus *Gentiana*. SCHUSTLER (1923) has also clearly pointed out that the only characteristic in common for these two genera are the jointed corolla lobes, and SMITH (in HYLANDER, 1945) has given a number of good distinctive characters separating the two groups. Nevertheless, both genera are to be regarded as rather heterogeneous from the point of view of cytotaxonomy, although the majority of the sections of *Gentianella* are characterized by the chromosome number $2n = 36$ and the basic number $x = 6$, and the chromosomes are between 1,5 and 5,0 micra long.

The two sections *Comastoma* and *Crossopetalum* differ from the other subgroups of *Gentianella* not only in their morphological characteristics, but also in their basic number of chromosomes. From a morphological point of view, WETTSTEIN (1896) suggested that the section *Comastoma* ($x = 5$) might be more correctly placed close to or even within the genus *Lomatogonium*. According to LINDSEY (1937), the floral anatomy of this genus indicates that it should he regarded as one of the most primitive groups of the family. This is supported by the fact that the chromosome number of *L. rotatum* is diploid with the basic number $x = 5$.

The section *Crossopetalum*, however, being clearly distinct from the section *Comastoma* and the other subgroups of *Gentianella* as well, includes two groups, which on cytological basis can hardly be regarded as very closely related. One of these groups, comprising *G. ciliata* and *G. detonsa* with the tetraploid number $2n = 44$ of the basic number $x = 11$, is morphologically very well distinguishable from the other one, embracing *G. crinita* and *G. procera* with $2n = 78$, or the hexaploid number of $x = 13$. As LINDSEY (1940) has pointed out, the floral anatomy of this section also differs quite evidently from that of the other subdivisions of the genus. From an evolutionary standpoint it is very unlikely that both these groups represent the same ancestral line,

TABLE 1. *Chromosome number and size in Gentianaceae—Gentianinae.*

Species	2*n*	Author	Chromosome length
Gentiana L.			
sect. *Coelanthe* GRISEB.	(*x* = 5)		
G. lutea L.	40	FAVARGER, 1949, 1952	2,2—2,8 micra
G. punctata L.	40	FAVARGER, 1952; SKALINSKA, 1952	2,2—2,8 »
G. purpurea L.	40	FAVARGER, 1949	2,2—2,8 »
sect. *Pneumonanthe* NECK.	(*x* = 11, 13)		
G. asclepiadea L.	44	RORK, 1946, 1949; FAVARGER, 1949; SKALINSKA, 1952	2—3 »
G. Pneumonanthe L.	26	SCHEERER, 1939	3—5 »
G. Andrewsii GRISEB.	26	RORK, 1946, 1949	3—5 »
G. cherokeensis (LEM.) FERN.	26	RORK, 1946, 1949	3—5 »
G. clausa RAF.	26	RORK, 1946, 1949	3—5 »
G. decora POLL.	26	RORK, 1946, 1949	3—5 »
G. Gebleri FISCH.	26	SOKOLOVSKAJA and STRELKOVA, 1938	—
G. Makinoniana KUSNEZ.	26	SAKAI, 1934	—
G. Porphyrio J. F. GMEL.	26	RORK, 1946, 1949	3—5 micra
G. linearis FROEL.	26	RORK, 1946, 1949	3—5 »
G. puberula MICHX.	26	RORK, 1946, 1949	3—5 »
G. Saponaria L.	26	RORK, 1946, 1949	3—5 »
G. septenfida PALL.	26	SOKOLOVSKAJA and STRELKOVA, 1938	—
G. villosa L.	26	RORK, 1946, 1949	—
sect. *Frigida* KUSNEZ.	(*x* = 6, 13?)		
G. algida PALL.	26	SOKOLOVSKAJA and STRELKOVA, 1938	—
G. frigida HAENKE	24	SKALINSKA, 1952	3—5 micra
sect. *Aptera* KUSNEZ.	(x = 7, 13)		
G. crusiata L.	52	RORK, 1946, 1949; SKALINSKA, 1952	3—5 »
	51—52	FAVARGER, 1949	
G. dahurica FISCH.	26	RORK, 1946, 1949	3—4 micra
G. gracilipes TURRILL	26	RORK, 1946, 1949	2—3 »
G. Fetisowi RGL. et WNKL.	32—36	WOYCICKI, 1933	—
G. macrophylla PALL.	42	RORK, 1946, 1949	2—3 micra
G. phlogifolia SCH. et KY.	52	RORK, 1946, 1949	3—4 »
G. straminea MAXIM.	52	RORK, 1946, 1949	2—3 »
sect. *Chondrophylla* BG.	(*x* = ?)		
G. altaica PALL.	26	SOKOLOVSKAJA and STRELKOVA, 1938	—
G. prostrata HAENKE	c. 36	FAVARGER, 1952	2—4 micra

Species	2n	Author	Chromosome length
sect. *Thylacites* REN.	($x = 6$)		
G. acaulis L.	36	RORK, 1946, 1949	2,5—4,5 micra
G. alpina VILL.	36	FAVARGER, 1949	2,5—4,5 »
G. Clusii PERR. et SONG.	36	FAVARGER, 1949; SKALINSKA, 1952	2,5—4,5 »
G. Kochiana PERR. et SONG	36	FAVARGER, 1949	2,5—4,5 »
sect. *Cyclostigma* GRISEB.	($x = 7, 11$)		
G. nivalis L.	14	FAVARGER, 1949; KNABEN, 1950; D. LÖVE, 1953	2—4 »
G. verna L.	28	FAVARGER, 1949; SKALINSKA, 1952; MATTICK in TISCHLER, 1950	2—4 »
G. bavarica L.	28	MATTICK in TISCHLER, 1950	—
G. brachyphylla VILL.	24	MATTICK in TISCHLER, 1950	—
G. utriculosa L.	22	FAVARGER, 1952	2—3 micra
Gentianella MOENCH.			
sect. *Andicola* GRISEB.	($x = 6$)		
G. saxosa (FORST.)	36	FAVARGER, 1952	2—4 »
sect. *Endotricha* FROEL.	($x = 6$)		
G. Amarella (L.) H. SM.	36	D. LÖVE, 1953	2—4 »
G. austriaca (A. et J. KERN.)	36	FAVARGER, 1952	2—4 »
G. campestris (L.) H. SM.	36	FAVARGER, 1949; D. LÖVE, 1953	2—4 »
G. insubrica (KUNZ.)	36	FAVARGER, 1952	2—3 »
G. praecox (KERN.)	36	SKALINSKA, 1952	2—4 »
sect. *Antarctophila* GRISEB.	($x = 6$)		
G. tenuifolia PETRIE	36	FAVARGER, 1952	2,5—5 »
sect. *Arctophila* GRISEB.	($x = 6$)		
G. aurea (L.) H. SM.	36	D. LÖVE, 1953	2—4 »
G. quinquefolia (L.) D. LÖVE	36	RORK, 1946, 1949	2—4 »
sect. *Crossopetalum* FROEL.	($x = 11, 13$)		
G. ciliata (L.)	44	FAVARGER, 1949	3—6 »
G. detonsa (ROTTB.) G. DON	44	D. LÖVE, 1953	4—7 »
G. crinita (FROEL.)	78	RORK, 1946, 1949	2—3 »
G. procera (HOLM.)	78	RORK, 1946, 1949	2—3 »
sect. *Comastoma* WETTST.	($x = 5$)		
G. tenella (ROTTB.) H. SM.	10	FAVARGER, 1949; KNABEN, 1950; D. LÖVE, 1953	2—4 »
Lomatogonium A. BR.	($x = 5$)		
L. rotatum (L.) A. BR.	10	D. LÖVE, 1953	2—4 »
Frasera WALT.	($x = 13$)		
F. caroliniensis WALT.	78	RORK, 1946, 1949	2—3 »
Swertia L.	($x = ?$)		
S. perennis L.	28	FAVARGER, 1952	2—4 »
S. cuspidata (MAXIM.) KITAGAWA	18	SAKAI, 1940	—
Halenia BORKH.	($x = 11$)		
H. elliptica D. DON.	22	FAVARGER, 1952	2—4 micra

and thus cannot be included in the same section of the genus. If they were excluded from the genus *Gentianella* at the same time as the section *Comastoma*, this would result in a much greater conformity of the genus from a cytotaxonomical viewpoint. On the other hand, the step taken by NECKER (1790) and amended by RAFINESQUE (1836) and RYDBERG (1906 a, b, 1913, 1917, 1932) separating the whole section *Crossopetalum* as the genus *Anthopogon* NECKER, is not sufficient to get a complete agreement of the morphological and evolutionary data. This can only be done if the genus *Anthopogon* is defined in its strict sense including *G. ciliata*, *G. detonsa* and their allies with $x = 11$ at the same time as the group of *G. crinita*, *G. procera* and their relatives with $x = 13$ are placed in a separate genus.

As regards the genus *Gentiana* in the sense given in Table 1 of the present paper, also this is found to have the same diversity in the basic numbers of chromosomes as the *Gentianella* groups. By aid of the chromosome morphology and the basic numbers, the genus can be divided into five groups, with $x = 5$, 6, 7, 11, and 13 respectively.

The lowest number, $x = 5$, is typical for the section *Coelanthe*, with only octoploid species. Their chromosomes are small.

The number $x = 6$ seems to be typical for the section *Thylacites* as well as for some members of the *Aptera* section. The chromosomes of these species are medium long, or varying between 1,0 and 4,5 micra.

The basic number $x = 7$ is characteristic of at least most of the species of the section *Cyclostigma*, which is also characterized by chromosomes of varying sizes, from 2 to about 4 micra long in the same cell.

The basic number $x = 11$ is known only from the species *G. asclepiadea*, which is characterized by $2n = 44$ chromosomes, most of which are small, a few pairs only being long. Although this species is usually regarded as a member of the *Pneumonanthe* section, this is not in agreement with the cytological observations, as all the other species of this group are known as diploids with the basic number $x = 13$ and have mainly long and slender chromosomes.

Section *Frigida* is still not sufficiently known cytologically, but it is likely that its basic chromosome number is $x = 6$, as SKALINSKA (1952) has definitely found *G. frigida* to have $2n = 24$. The number $2n = 26$ for *G. algida* by SOKOLOVSKAJA and STRELKOVA (1938) either indicates that the two species do not belong to the same section, or, more probably, that they have counted 2 chromosomes too many, as the two species are taxonomically very closely related.

Section *Chondrophylla* also requires a revision as, in this section, we know only two species with two very different numbers, *G. prostrata* with $2n = 36$, and *G. altaica* with $2n = 26$ chromosomes.

If the above-mentioned groups should be given generic names as suggested for those of *Gentianella s. lat.*, it is quite evident that the section *Coelanthe* with $x = 5$ chromosomes represents the genus *Gentiana* in its strictest sense, as it includes the type species of the genus, *G. lutea* L. The sections with $x = 13$, or *Aptera p. p.*, *Frigida*, and *Pneumonanthe* excl. *G. asclepiadea*, should form the genus *Dasystephana* ADANSON (1763) in its strict sense, while the groups with $x = 6$, 7, and 11, respectively, do not seem to have been given generic names by previous taxonomists.

The above discussion should be looked upon as rather hypothetical, although it clearly indicates that the family Gentianaceae can be regarded as one of the most interesting cytotaxonomical objects hitherto known. Its evolution at the species level seems to have been based on the formation of abrupt-species by aid of polyploidy as well as on the gradual evolution of species with the same chromosome number. The generic diversification, however, has been based in a high degree on alloploidy, as shown in the great variation in the basic numbers of chromosomes between the different groups.

SUMMARY.

(1) The chromosome numbers of seven species of Icelandic Gentianaceae are reported. New numbers are: $2n = 36$ for *Gentianella Amarella* and *G. aurea*, $2n = 44$ for *G. detonsa*, and $2n = 10$ for *Lomatogonium rotatum*. The numbers for *Gentiana nivalis* ($2n = 14$), *Gentianella campestris* ($2n = 36$), and *G. tenella* ($2n = 10$), coincide with previous reports.

(2) All chromosome numbers known within the family are listed. It is shown that different basic numbers are met with in different sections, and that on basis of these numbers as well as on chromosome morphology, the separation of *Gentiana* and *Gentianella* as two genera only is insufficient. Different taxonomical changes in order to get generic uniformity are discussed.

(3) The evolution of Gentianaceae at the generic level has been based on alloploidy, while the evolution at the species level is based on abrupt as well as gradual formation of sterility barriers.

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CHANGE OF DOMINANCE IN DIFFERENT GENIC ENVIRONMENTS

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THE problem of dominance is of secondary interest in genetics, since dominance is probably caused by different phenotypical factors, anatomical or chemical and physical. It is well known that the dominance of a given gene may change with changes in the external environment. But also changes in the genic environment, caused by the interactions of the genes, may result in changes in the expression and also the dominance of a given gene. Cases where a change in dominance is caused by the genic environment are, therefore, of a certain interest; two such cases are here reported. ERNST NILSSON (1929) has reported a similar case.

Change of dominance in a flower colour gene in Lupinus angustifolius. — In a paper in volume II of Hereditas (HALLQVIST, 1921) the present author has reported the inheritance of flower colour in *Lupinus angustifolius*. The following four genes were found to influence the flower colour:

B: blueish red,
V: purple,
BV: blue,
R: pure red, without any blueish tint. This gene is also a fundamental colour gene, *rr* plants are white.
F: full colour intensity, *ff* plants are dilution forms of the colours determined by the other genes.

As is generally the case with *albina* types, the *rr* plants reported in the paper quoted were completely without anthocyanin in all vegetative parts, including the sepals. At first also the petals were pure white but on ageing they developed a colour which was always quite evident and sometimes fairly strong. This phenomenon is typical of the genus *Lupinus* where all flower colours become darker with age. The white plants of this type were not true *albinas* but might be classified as *pseudo-albina.*

In further experiments I found a type without age colouring, com-

pletely free from any trace of anthocyanin in all parts of the plant; a true *albina*. It occurred in the following combination:

Weak red colour × White with age colouring
RRff *rrFF*

and the true *albina* was probably constituted *rrff*.

Both *R* and *F* are dominant and the expected segregation in F_2 should be: 9 strongly coloured : 3 weakly coloured : 3 white with age colouring : 1 pure white. Five F_2 families gave the following result:

	strong colour	weak colour	*pseudo-albina*	*albina*
found	126	37	23	39
expected	126,6	42,2	42,2	14,1
χ^2	0,00	0,64	8,74	43,97 = 53,35 P < 0,001

The agreement is very poor and there is evidently some disturbance. Linkage is excluded since it should have resulted in a surplus of parental types; instead, the double recessive occurs in excess, the double dominant in normal proportions.

The disturbance occurs only within the *rr*-group. According to earlier results, the 62 *rr* plants should have given $^3/_4$ *pseudo-albina*, *i. e.* 46,5 *pseudo-albina* and 15,5 true *albina*. The actual proportions are nearly reverse, *i. e.* 23 *pseudo-* and 39 true *albina*. This result may be fully explained by the hypothesis that the usual dominance in the *F* gene, $F > f$, has become reverse in the presence of homozygously recessive *rr*. If such is the case, the dihybrid segregation should be 9 : 3 : 1 : 3 in F_2 or:

	strong colour	weak colour	*pseudo-albina*	*albina*
expected	126,6	42,2	14,1	42,2
found	126	37	23	39
χ^2	0,00	0,64	5,62	0,24 = 6,50 P > 0,05

The agreement is fairly good with this hypothesis.

Change of dominance in chlorophyll genes in barley. — In earlier papers in volumes IV, V and VIII of Hereditas the author (HALLQVIST, 1923, 1924, 1926—27) has reported the occurrence and segregation of a number of chlorophyll mutants in barley. One group of such mutants has been named *virescens*, in which the seedlings are at first yellowish white and later become light green in the tips of the leaves.

Diallel crosses have been made between three such types, *viz. virescens* I, II and III. In all three crosses the double recessive homozygote is completely devoid of chlorophyll; it is as pure white as the *albina* mutants. At the same time the leaf is as narrow as that of true *albina* mutant and the bifactorial combination of two *virescens* genes, thus, phenotypically completely imitates a monofactorial *albina* mutant.

In dihybrid segregations of two *virescens* it is often difficult to classify the two genes and both *virescens* are therefore usually classified together. In a normal, undisturbed segregation the expected proportions are, thus, 9 green : 6 *virescens* : 1 white.

In the cross *virescens* I × *virescens* III these proportions are realized.

	green	*virescens*	white
found	3668	2364	387
expected	3610,7	2407,2	401,2
χ^2	0,91	0,78	0,50 = 2,19
			P = 0,50—0,30

The agreement is very good.

When *virescens* II is included in the cross the results are quite different.

	green	*virescens*	white
	Virescens I × *virescens II*		
found	144	72	46
expected	147,4	98,3	16,4
χ^2	0,08	7,04	53,42 = 60,54
			P < 0,001
	Virescens II × *Virescens III*		
found	12774	5629	4143
expected	12682,1	8454,8	1409,1
χ^2	0,67	944,5	5305 = 6250,17
			P < 0,001

In both cases the number of green plants is quite normal, while there are about 3 times as many white ones as expected, with a corresponding deficit in *virescens*.

As was the case in the lupine, linkage is excluded as an explanation. Most probably the phenotypically white group has a different genotypical composition from the one expected, and the most reasonable hypothesis is that recessive homozygosity in V_{II} results in a change in dominance in the pairs V_I—v_I and V_{III}—v_{III}. Phenotypical *albina* should then occur not only in the two double recessives $v_{II}v_{II}v_Iv_I$

and $v_{II}v_{II}v_{III}v_{III}$ but also in the biotypes $v_{II}v_{II}V_{I}v_{I}$ and $v_{II}v_{II}V_{III}v_{III}$, and the segregation should be 9 : 4 : 3 instead of 9 : 6 : 1. According to this hypothesis, expected numbers and χ^2 in the two crosses should be

	green	*virescens*	white
	Virescens I × *virescens II*		
expected	147,4	65,6	49,2
χ^2	0,08	0,99	0,21 = 1,18
	Virescens II × *virescens III*		
expected	12681,1	5636,4	4227,3
χ^2	0,67	0,01	1,68 = 2,36

In both cases the agreement is very good.

There is one further reason to accept the hypothesis. In the cross *virescens* II × *virescens* III the *albina* group is not quite uniform. There are numerous plants with the colour and narrow leaf of *albina* which have, however, a very minute green spot at the tip of the leaf. It is reasonable to suppose that these plants are the $V_{III}v_{III}$ plants which because of the change in dominance have become *albina* but with a very small effect of the V_{III} allele. If this is true, 2762 of the 4143 plants classified as *albina* should have shown this minute green spot; it was observed in only 2573 plants, however. The deficiency may be due to difficulties in classification, since the very small green spot is rather difficult to observe; it may also be due to a weak penetration of the single V_{III} allele.

There is a striking similarity between the two cases here reported. In both cases recessive homozygosity in one gene has resulted in a change in dominance in another pair of alleles. In lupine the constitution *rr* had the result that the *Ff* plants became phenotypically similar to *ff* plants. In barley the combination $v_{II}v_{II}V_{I}v_{I}$ has exactly the same *albina* phenotype which else is typical of double recessives in *virescens* genes. In $v_{II}v_{II}V_{III}v_{III}$ the change towards *albina* is not complete, as the single V_{III} allele may be traced in most cases by a minute green spot, but the dominance for V_{III} has changed into a strong prevalence for the other allele, v_{III}.

It is interesting to note that in both cases the genes involved have a reducing effect. Both *r* and *f* reduce the anthocyanin colouring in lupine; v_{I}, v_{II} and v_{III} all reduce the chlorophyll in the plastids in barley. The reducing effect of the genes *r* and v_{II}, respectively, may be

especially strong, which might explain their effect in changing the dominance of other genes. When the two genes mentioned are homozygously recessive the colour is already so reduced that the change from normal to mutant of one single further allele in another gene is sufficient to result in a complete absence of the colour. Usually and in other genic environments a corresponding change of one allele of the latter genes has no outwardly visible effect.

The above recorded experiments were carried out as early as in 1925—1928 at the Weibullsholm Plant Breeding Institute, Landskrona.

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INCREASED CROSSABILITY IN LAMIUM AFTER CHROMOSOME DOUBLING

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INTRODUCTION.

IN Northern Europe four annual *Lamium* species occur, two of which, *L. hybridum* VILL. and *L. intermedium* FR., are more or less intermediary, from a morphological point of view, between the other ones, *L. purpureum* L. and *L. amplexicaule* L. Especially *L. intermedium* has often been regarded as a hybrid between the two last-mentioned species. Several attempts have been made to find out experimentally the relationships between the different species; a review of these as well as a close account of the species has been published (BERNSTRÖM, 1952, and in press). From this will be seen that *L. purpureum* and *L. amplexicaule* are diploid ($2n = 18$), while *L. hybridum* and *L. intermedium* are tetraploid ($2n = 36$). MÜNTZING (1926) and JÖRGENSEN (1927) have found that the only hybrids to be obtained between the different species are those between *L. hybridum* and *L. amplexicaule*.

In an attempt to increase the possibilities of hybridization between the species, thereby enabling a closer examination of their relationships, autotetraploids have been produced in *L. purpureum* and *L. amplexicaule*, and have been used in comprehensive hybridization experiments between the species. The experiments have been based on the well-known fact that the crossability between species often depends on their chromosome numbers; thus, a change of the chromosome number of one component may render an otherwise failing cross combination possible. Preliminary reports have been published on results of the cross experiments in *Lamium* (BERNSTRÖM, 1944, 1949). In the present paper a comprehensive account will be given of all the cross experiments made between species as well as within species between multiple chromosome numbers. The cross-pollination method adopted as well as the cultivation conditions, *etc.*, have been described earlier (BERNSTRÖM, 1952).

It has been possible to demonstrate, as a result of the experiments, that *L. intermedium* is an allopolyploid between *L. purpureum* and *L. amplexicaule*, and that *L. hybridum* is also an allopolyploid. Prob-

ably, the parent species of *L. hybridum* are *L. purpureum* and another species which may be supposed to be *L. bifidum* CYR. (BERNSTRÖM, in press, and in a later paper).

EXPERIMENTAL RESULTS.

In Table 1 all cross-pollinations between species are recorded, except those in which the styles were shortened (BERNSTRÖM, 1944). By estimating the degree of seed-setting after cross-pollination, the number of hybrid seeds obtained has been estimated on the percentage of hybrids among the plants obtained, the number of hybrid seeds being expressed in percentage of the number of cross-pollinated ovaries which are 4 per flower. The difference between the number of seeds obtained and of plants obtained is often rather great, owing to the fact that part of the seeds were empty or contained embryos which were more or less aborted; further, part of the seeds did not germinate in spite of the seed-coats being removed, or were damaged by this treatment, *etc.* Probably, the estimated percentages of seed-setting are, as a rule, a little too low, since it may be presumed that a comparatively larger number of those seeds having small or otherwise non-germinating embryos are hybrid ones than seeds from unintentional self-pollination.

Generally, the results of cross-pollinations in the summer are considerably superior to those in the autumn; in fact, results are often attained only in the summer. Therefore, a distinction has been made in Table 1 between cross-pollinations made in summer and in autumn, respectively. However, as cross-pollination experiments have been repeated in a great number of the hybrid combinations for several years, the flowering furthermore taking place in rather different times of the season and the weather conditions being variable, the difference between summer and autumn has been blurred in Table 1. Thus, some of the cross combinations show about the same degree of seed-setting in autumn as in summer. However, among these is, without exception, the autumn generation in 1943, and this was a particularly early one; at the same time, the weather was warm and sunny when the cross-pollinations were carried out. By summer is meant the time from the last days of May until the end of August, while the autumn has been taken to extend from the end of August to the end of October. In spite of the disadvantages mentioned above, the approximate possible degree of seed-setting of the various cross combinations is more clearly de-

monstrated by distinguishing in Table 1 between the values for summer and autumn than by combining them. It should be pointed out that the species studied are weeds which begin flowering in the spring and continue until late in the autumn, setting seed almost as well at that time as in summer.

Turning now to the results of the cross-pollination experiments, it may firstly be established that *L. hybridum* × *L. amplexicaule* is still the only combination obtainable of those attempted with the original chromosome numbers. Thus, the tetraploid species *L. intermedium* and *L. hybridum* are not able to hybridize in spite of extensive attempts, and in spite of the fact that two artificial lines of *L. intermedium* have also been used besides those occurring in nature. As has been demonstrated elsewhere (BERNSTRÖM, in press), the latter are probably almost identical.

On the other hand, it is possible to produce the following 4 new species hybrids by using tetraploid forms of *L. purpureum* and *L. amplexicaule*, respectively, instead of diploid: tetraploid *L. purpureum* × diploid *L. amplexicaule*, tetraploid *L. amplexicaule* × *L. intermedium* (and reciprocally), *L. intermedium* × tetraploid *L. purpureum*, and *L. hybridum* × tetraploid *L. purpureum* (and reciprocally). Thus, the doubling of the chromosome number of the two diploid species has now made it possible to produce hybrids in all those combinations of which the originally diploid species form part (*i. e.*, all combinations possible except *L. hybridum* × *L. intermedium*).

Some lines of two different, artificially produced allopolyploids were also included in the cross experiments. The one was obtained by colchicine treatment of hybrids between *L. hybridum* and *L. amplexicaule*, and should thus be labelled 6*x* (4*x hybridum* × 2*x amplexicaule*), while the other originated in the same way from hybrids between tetraploid *L. purpureum* and diploid *L. amplexicaule*; consequently, this one can be written 6*x* (4*x purpureum* × 2*x amplexicaule*). For the sake of simplicity, they are called *L. hybrido-amplexicaule* and *L. purpureo-amplexicaule*, respectively. The purpose with *L. hybrido-amplexicaule* was, *i. a.*, to produce tetraploid hybrids between *L. hybridum* and *L. amplexicaule* in an indirect way by crossing the hexaploid form with diploid *L. amplexicaule*, as it has proved impossible to obtain the tetraploid hybrids directly (*cf.* Table 1). However, the attempt was unsuccessful. Nor is it possible to hybridize *L. purpureo-amplexicaule* with diploid *L. amplexicaule*, which is to

TABLE 1. *The results of cross-pollinations between species, including synthesized forms, of Lamium.*

Cross combinations	Season	Number of flowers pollinated	Number of seeds obtained	Number of plants obtained	Number of hybrids obtained	% seed-setting of hybrid seeds	Number of cross combinations tried	Number of lines used [2]		Years
								♀	♂	
2x purp.×*2x amplex.*	summer	48	0	0	0	0	1	1	1	48
2x amplex.×*4x purp.*	»	33	0	0	0	0	3	2	2	43
4x purp.×*2x amplex.*	»	963	358	207	197	9	14	7	4	43—49
	autumn	247	0	0	0	0	3	3	2	44
2x purp.×*4x amplex.*	summer	21	0	0	0	0	3	2	2	43
4x amplex.×*2x purp.*	»	97	1	0	0	0	6	4	5	43, 45
4x purp.×*4x amplex.*	»	392	7	6	0	0	11	4	7	41—43, 45, 46
	autumn	17	0	0	0	0	1	1	1	42
4x amplex.×*4x purp.*	summer	196	5	1	0	0	8	5	5	41—43, 45
2x purp.×*4x interm.*	summer	30	0	0	0	0	1	1	1	46
4x interm.×*2x purp.*	»	214	1	1	0	0	2	2	2	46
4x purp.×*4x interm.*	»	99	2	1	0	0	5	4	3	42—45
	autumn	44	0	0	0	0	2	1	2	42
4x interm.×*4x purp.*	summer	574	40	20	5	< 1	9	5	6	42—45, 48
	autumn	213	3	3	0	0	4	3	3	42, 44
4x purp.×*4x hybr.*	summer	139	27	7 [1]	7	5	8	4	5	42—43, 48
	autumn	39	0	0	0	0	3	2	2	42—43
4x hybr.×*4x purp.*	summer	2160	1555	656 [1]	588	16	25	7	4	42—45, 47—49
	autumn	365	153	99	94	10	7	7	7	43—45
8x hybr.×*4x purp.*	summer	212	1	1	0	0	2	1	2	48—49
4x interm.×*2x amplex.*	summer	60	0	0	0	0	6	2	3	46
4x amplex.×*4x interm.*	»	28	27	25	25	24	2	2	1	43
	autumn	35	51	27 [1]	27	36	2	2	1	43
4x interm.×*4x amplex.*	summer	80	48	22	18	12	5	3	4	43, 46
	autumn	139	62	39	36	10	4	3	2	42—44
2x amplex.×*4x hybr.*	summer	17	6	3	3	9	2	1	2	43
	autumn	16	0	0	0	0	1	1	1	43

	autumn	139	62	39	36	10	4	3	2	42—44
2x amplex.×*4x hybr.*	summer	17	6	3	3	9	2	1	2	43
	autumn	16	0	0	0	0	1	1	1	43
4x hybr.×*2x amplex.*	summer	174	413	175 [1]	173	59	9	4	4	43—44, 47
	autumn	18	52	46	42	66	1	1	1	43
4x amplex.×*4x hybr.*	summer	64	3	1	0	0	6	3	3	42—43
	autumn	49	0	0	0	0	3	2	2	43
4x hybr.×*4x amplex.*	summer	317	18	13	0	0	13	5	7	42—46
	autumn	26	1	0	0	0	1	1	1	42
4x hybr.×*4x interm.*	summer	258	3	1	0	0	7	5	3	46, 48
4x interm.×*4x hybr.*	»	1334	7	5	0	0	24	7 [3]	7	46—50
6x (*4x hybr.*×*2x amplex.*)×	summer	83	2	2	0	0	5	1	5	45—46
×*2x purp.*	autumn	62	0	0	0	0	2	1	2	44
6x (*4x hybr.*×*2x amplex.*)×	summer	193	1	1	0	0	4	1	4	45—47
×*2x amplex.*	autumn	46	0	0	0	0	1	1	1	44
6x (*4x hybr.*×*2x amplex.*)× ×*4x amplex.*	summer	68	27	24	24	10	3	1	3	45—46
6x (*4x hybr.*×*2x amplex.*)× ×*4x interm.*	»	256	46	23	23	4	3	3	2	48—49
6x (*4x purp.*×*2x amplex.*)× ×*2x amplex.*	summer	69	0	0	0	0	1	1	1	48
6x (*4x purp.*×*2x amplex.*)× ×*4x amplex.*	»	152	0	0	0	0	2	2	2	48—49
6x (*4x purp.*×*2x amplex.*)× ×*4x interm.*	»	259	2	0	0	0	3	2	2	48—49
6x (*4x purp.*×*2x amplex.*)× ×*4x hybr.*	»	160	0	0	0	0	2	1	2	48—49
6x (*4x purp.*×*2x amplex.*)× ×*6x* (*4x hybr.*×*2x amplex.*)	summer	90	2	1	1	< 1	1	1	1	49
6x (*4x hybr.*×*2x amplex.*)× ×*6x* (*4x purp.*×*2x amplex.*)	»	97	6	2	2	2	1	1	1	49

[1] The number of plants cultivated was lower than that of available seedlings, in one or several generations. [2] Hybrids between lines when occasionally used as parents are not specifically counted. [3] Including 2 different artificial lines.

TABLE 2. *Results of cross pollinations between autotetraploid lines and between forms with different chromosome numbers within species of Lamium.*

Cross combinations	Season	Number of flowers pollinated	Number of seeds obtained	Number of plants obtained	Number of hybrids obtained	% seed-setting of hybrid seeds	Number of cross combinations tried [2]	Number of lines used [3] ♀	Number of lines used [3] ♂	Years
4x purp.×*2x purp.*	summer	126	1	0	0	0	6	3	5	44—45
	autumn	19	0	0	0	0	1	1	1	43
2x purp.×*4x purp.*	summer	38	0	0	0	0	1	1	1	45
	autumn	11	0	0	0	0	1	1	1	43
4x purp.×*4x purp.*	summer	388	218	119 [1]	116	14	12	7	7	44—49
	autumn	109	3	2	1	< 1	8	5	5	44
8x purp.×*4x purp.*	summer	221	0	0	0	0	3	1	3	49—50
4x amplex.×*2x amplex.*	summer	64	0	0	0	0	5	3	3	44
	autumn	22	0	0	0	0	3	2	2	43
2x amplex.×*4x amplex.*	»	27	0	0	0	0	3	2	2	43
4x amplex.×*4x amplex.*	summer	281	112	55 [1]	55	10	15	6	6	44—45
	autumn	52	25	10	10	12	4	2	3	44
8x hybr.×*4x hybr.*	summer	695	2	1	1	< 1	8	3	5	46—49
4x hybr.×*8x hybr.*	autumn	26	0	0	0	0	1	1	1	45

[1] The number of plants cultivated was lower than that of available seedlings, in one or several generations.
[2] Reciprocal combinations have been counted separately.
[3] Hybrids between lines when occasionally used as parents are not specifically counted.

be regretted, because it would have meant a direct synthesis of *L. intermedium*.

Table 2 has been included in order to make possible, *i. a.*, an estimation of the easiness by which the compatible combinations can be realized. It has been arranged in the same way as Table 1. A comparison between the tables shows the seed-setting in some of the hybrid combinations to be just about as good as the seed-setting subsequent to intraspecific pollination within the mother species. This holds true, *e. g.*, for tetraploid *L. amplexicaule* × *L. intermedium* and tetraploid *L. purpureum* × diploid *L. amplexicaule*. When cross-pollinated with diploid *L. amplexicaule*, *L. hybridum* too sets seed as well as after intraspecific cross-pollination (*cf.* BERNSTRÖM, 1952, Table 1). On the other hand, the seed-setting is far poorer in several other hybrid combinations, to a varying degree. We will return to this below.

The seed-setting has, further, turned out to be different in one and the same hybrid combination, depending on the choice of parent lines. This is very clearly demonstrated by cross-pollinations between tetraploid *L. purpureum* and diploid *L. amplexicaule*, so far as cross-combinations allowing comparisons have been tried; *cf.* Table 3. Thus, when pollinated with the *L. amplexicaule* line *a*8, the line *p*43 is characterized by a seed-setting considerably inferior to that of the line *p*7. Another example is presented by the tetraploid line *p*42, which, when pollinated in one generation with *a*8 and in another with *a*13, sets seed as well as the combination *p*7 × *a*8. However, while the seeds of *p*7 × *a*8 had well developed embryos in both generations, the seeds

TABLE 3. *Some results of cross-pollinations between tetraploid L. purpureum and diploid L. amplexicaule* (*cf. the text*).

Cross combinations	Year	Number of flowers pollinated	Number of seeds obtained	% seed-setting of hybrid seeds
*p*7×*a*8	1945	25	14	14
*p*42×*a*8	(summer)	30	17	14
*p*7×*a*8	1946	111	120	27
*p*42×*a*13	(summer)	51	50	25
*p*7×*a*8	1947	93	90	24
*p*43×*a*8	(summer)	60	2	1
*p*7×*a*13	1949	78	3	1
*p*42×*a*8	(summer)	72	23	8

of *p*42 × *a*13 were as good as these, but those of *p*42 × *a*8 were mostly empty with very small, discoloured embryos which were viable in only 2 seeds out of 17. In a later generation, too, the seed-setting was good in *p*42 when pollinated with *a*8, but the embryos in most of the seeds were too small to be viable. At the same time, the crossing *p*7 × *a*13 demonstrated a far inferior seed-setting than could be expected judging from the results of cross-pollinations between *p*7 and *a*8 in several generations. The results suggest a better compatibility in the latter combination than between *p*7 and *a*13.

The crossability between tetraploid *L. purpureum* and diploid *L. amplexicaule* thus depends a great deal on the parent lines used, and the result of the cross-pollinations is influenced by the choice of both the *purpureum* and the *amplexicaule* line. Another example of the importance of the choice of line for the compatibility is afforded within the combination tetraploid *L. purpureum* × *L. hybridum*. In spite of rather comprehensive cross experiments in three years, hybrid seeds have been obtained from one combination only, the percentage of seed-setting being then, however, as high as 16. Another cross combination did not yield any seed at the same time, as will be seen from the following figures:

Cross combination	Number of flowers pollinated	Number of seeds obtained
*p*61 × *h*37	33	0
*p*62 × *h*37	43	27

7 plants cultivated were all hybrids. As will be seen, the different results depend on the choice of the *L. purpureum* line. A great number of ovaries in *p*61 × *h*37 had increased in size, showing that fertilization had occurred.

In almost all cross combinations was observed, on harvesting the cross-pollinated flowers, that part of the ovaries have often increased more or less in size, even when no ripe seeds were obtained. In order to get an idea of the size of the embryos which obviously must exist, the young seeds from such enlarged ovaries in several hybrid combinations have been dissected at a magnification of 30 ×. From 10 to 30 days, mostly about 25, had passed after the pollination. It was a characteristic feature for most of the enlarged ovaries, which were distinguishable also by their more opaque appearance, that the ovules

filled up the greater part of them; in the ovaries externally unchanged, the ovules occupy about the third part. Most often, the more or less enlarged endosperms could also be seen, preserved or indicated by the shape of the endothelium, while the embryosacs in unchanged ovules could not be clearly observed. By dissection it was possible to lay bare the embryos in some of the cross combinations, while in others they were too small to be detected.

The endosperms and embryos were preserved in 70 % alcohol, and were afterwards transferred to glycerine, and measured. It became evident, and more clearly so in glycerine than in water or alcohol, that almost all the embryos were probably alive; most of them were white and firm, without any signs of decomposition, while occasionally more or less brown-coloured. In Table 4, in which the results of the measurements have been collected, those embryos are characterized as living which appeared as white and sound, or which were in any case only as little discoloured as those embryos in hybrid seeds which, although sometimes very small, were alive at the removal of the seed-coats and were able to develop (*cf.* BERNSTRÖM, 1952).

When judging Table 4, it must be kept in mind that the time of ripening of a seed after self-fertilization or intraspecific cross-pollination lasts 12—16 days in the diploid species, somewhat longer in the tetraploids, and up to 23—24 days after interspecific pollination. When no visible embryos have developed in, *e. g.*, *L. intermedium* × diploid *L. purpureum* even after 12 days, this quite obviously means that no embryos will develop to perceptible size after this time either. — The measurements have been made at a magnification of 45 ×.

Consequently, in some of the cross combinations embryos develop which are visible at the magnification used, and even rather well developed in spite of the fact that no ripe seeds have been obtained. (Seeds have been obtained only from *L. hybridum* × tetraploid *L. purpureum* and octoploid × tetraploid *L. hybridum* among the combinations embryologically examined; *cf.* Table 1.) There is a clear positive correlation between the average size of the endosperm and the existence of visible embryos in the material investigated, which is quite natural (*cf.* below). The differences in embryo size between the combinations are probably smaller in the table than they actually are, because the smallest embryos were evidently just too small to be seen and measured. Perhaps, too, the least developed embryos perished and disintegrated more rapidly than the greater ones, thereby escaping examination. The results nevertheless show that the differences in degree of

TABLE 4. *Size of endosperm with endothelium, and of embryos, particularly after crosses between species.*

(1 unit of measurement is equivalent to 0,033 mm.).

Cross combination	Number of cross combinations examined	Number of days between cross and harvest	Length of endosperm $\bar{x}$	Length of endosperm n	Length of embryo $\bar{x}$	Length of embryo n	Remarks
6*x* (4*x hybr.*×2*x amplex.*)××2*x amplex.*	1	28—30	8,0	38	10,2	28	25 embryos alive, but 4 seemed to be in a poor state. 13 of them were differentiated. The largest one measured 17,5 units
6*x* (4*x hybr.*×2*x amplex.*)××2*x purp.*	1	24—27	4,1	16	—	—	Endosperms in a very poor state. No embryos visible
4*x purp.*×4*x amplex.*	5	12—27	5,5	27	—	—	Endosperms mostly in a very poor state. No embryos visible
4*x hybr.*×4*x amplex.*	3	24—25	5,3	20	—	—	Endosperms in a very poor state. No embryos visible
4*x hybr.*×4*x purp.*	1	27	7,5	5	8,2	6	All embryos alive, but 2 of them seemed to be in a poor state. 2 of them were differentiated
4*x interm.*×2*x amplex.*	3	23	7,3	13	4,6	11	All embryos alive, but 4 seemed to be in a poor state. None was differentiated
4*x interm.*×2*x purp.*	1	10—12	3,9	25	—	—	No embryos visible
2*x purp.*×4*x interm.*	1	15	3,6	4	—	—	» » »
4*x hybr.*×4*x interm.*	3	24—25	6,6	31	—	—	» » »
4*x interm.*×4*x hybr.*	2	23	9,6	32	5,0	2	Both embryos alive, but undifferentiated, of the same length
8*x hybr.*×4*x hybr.*	2	26	7,0	14	5,8	8	All embryos alive; 2 of them differentiated. The largest embryo measured 12,0 units and the smallest one 3,5

embryo development are quite obvious between the combinations, even between those with one parent species in common, *e. g.*, *L. hybrido-amplexicaule* × diploid *L. amplexicaule* and diploid *L. purpureum*, respectively, and *L. intermedium* × diploid *L. amplexicaule* and diploid *L. purpureum*, respectively. Probably the table is not correct even as regards the difference between *L. hybridum* × *L. intermedium* and the reciprocal cross; it is probably greater than it appears to be, because often the endosperm is not visible when *L. hybridum* is the mother component, in spite of the fact that the young seed has increased in size in the usual way, indicating fertilization, while it is always visible in *L. intermedium* when pollinated with *L. hybridum*.

The greatest embryo in *L. hybrido-amplexicaule* × diploid *L. amplexicaule* was nearly 0,6 mm in length, *i. e.* a size equal to those of poorly developed embryos from fertile hybrid combinations, which sometimes have been able to germinate and develop into full-grown plants. The difference between the possibility of producing seeds between, *e. g.*, *L. intermedium* and tetraploid *L. purpureum* and the impossibility of obtaining seeds between *L. hybrido-amplexicaule* and diploid *L. amplexicaule* cannot, therefore, be very great; the boundary-line between possible and impossible cross combinations is obviously wavering, and depends on the extent of the cross-pollination experiments and the external conditions.

Finally, the swelled ovaries with enlarged ovules and more or less developed endosperms and embryos indicate, on the whole, that none of the so far sterile cross combinations in Table 4 is likely to have failed on account of non-fertilization. Investigations by COOPER and BRINK (1945), BRINK and COOPER (1947), HÅKANSSON (1952), and others, have thrown light upon the embryological processes leading to seed abortion in incompatible hybrid combinations. The central position of the endosperm in this connection has been emphasized, and also the fact that, as a rule, the embryo is starved to death when the external prerequisites for its development no longer exist. Investigations in *Galeopsis* (HÅKANSSON, 1952), a species related to *Lamium*, are in good accordance with investigations in other materials (*loc. cit.*), and observations in *Lamium* indicate similar conditions also in this genus; indications of this are the varying development of endosperms and embryos in different combinations, the correlation between endosperm and embryo size and, in particular, the fact that the embryos in incompatible combinations generally seem to be alive as late as about 25 days after fertilization.

DISCUSSION.

It is a well-known observation that the chromosome numbers are often of a great or even decisive importance for the result of cross-pollinations between plant species in genera with differing chromosome numbers. As a rule, it is difficult or even quite impossible to produce triploid hybrids between species with multiple chromosome numbers, or between a diploid and its autotetraploid. In both these cases, but particularly in the latter one, is the abortion of the ovules probably due to changes in the quantitative relations of the chromosome numbers between endosperm and maternal tissue, as supposed by WATKINS (1932) and MÜNTZING (1933) and embryologically elucidated by, *i. a.*, COOPER and BRINK (1945), BRINK and COOPER (1947), and HÅKANSSON (1952). There are, however, many exceptions where both autotriploids and allotriploids have easily been produced (*cf.* the papers cited above), and cases like these show that the presumed cause of the abortion is sometimes neutralized, and that, consequently, it might not always be taken for granted that it is the disturbed balance of chromosome numbers between endosperm and mother tissue which is in fact the final cause of the abortion of the triploid embryos.

In *Lamium*, too, the chromosome numbers play a decisive part as far as the crossability between the species, as well as between diploids and their autotetraploids, is concerned. It is interesting to note, however, that an autohexaploid plant of *L. hybridum* was attained. From the numbers of cross-pollinated flowers in Table 2 it may seem as if the success — small though it is — in hybridizing octoploid and tetraploid *L. hybridum*, as compared with the negative attempt to produce triploid *L. purpureum* and *L. amplexicaule*, is attributable to the very much larger number of cross-pollinated flowers in the first-mentioned species. Hexaploid embryos of *L. hybridum* are, however, capable of developing comparatively well (*cf.* Table 4), whereas the development of endosperms and embryos is quite insignificant in tetraploid *L. purpureum* and *L. amplexicaule* when pollinated with haploid pollen grains.

The numerical relationships between the chromosome numbers of the maternal tissue, the endosperm and the embryo, respectively, are equivalent for octoploid × tetraploid *L. hybridum* as for tetraploid × diploid *L. purpureum* and *L. amplexicaule*; cross combinations in *L. hybridum* are, however, distinguished from the latter by its double chromosome number, its allopolyploid nature and its own species

characteristics. Probably, a combination of all these qualities account for the better development after fertilization in *L. hybridum*. In any case, it is not likely to depend on the higher number of chromosomes only, as octoploid × tetraploid *L. purpureum* has failed, so far, and the ovaries swelled but to a small degree.

As regards the interspecific cross experiments, it is interesting to note — particularly in view of the intraspecific conditions dealt with above — that in some cases tetraploid *L. purpureum* is equally apt to hybridize with diploid *L. amplexicaule* as to set seed after intraspecific pollination (Tables 2 and 3), and that likewise the seed-setting in *L. hybridum* when pollinated with diploid *L. amplexicaule* is equally good as in *L. hybridum* when artificially pollinated by itself (Table 1 in the present paper and Table 1 in BERNSTRÖM, 1952). On the other hand, it is impossible, or at any rate difficult, to produce hybrids between tetraploid *L. amplexicaule* and diploid *L. purpureum* (the only seed obtained was empty), and further it is impossible to get tetraploid hybrids between *L. hybridum* and tetraploid *L. amplexicaule* as well as between tetraploid *L. purpureum* and tetraploid *L. amplexicaule*; these cannot, indeed, be hybridized as diploids either. Similarly, tetraploid *L. amplexicaule* is able to form hybrids with *L. intermedium*, and tetraploid *L. purpureum* with both *L. hybridum* and *L. intermedium*, whereas the corresponding triploid hybrids cannot be produced (MÜNTZING, 1926; JÖRGENSEN, 1927).

In this connection it may be observed, too, that *L. purpureum* and *L. amplexicaule* are incompatible when tetraploid as well as when diploid. There are examples, however, which may indicate compatibility between species after doubling of their chromosome numbers in contrast to the diploids being incompatible. Thus, FRANDSEN (1943) was not able to hybridize diploids of *Brassica nigra* and *Brassica campestris*, but only their tetraploid forms. Unfortunately, the number of diploid flowers cross-pollinated is not mentioned.

Thus, while triploid as well as tetraploid species hybrids can be produced without difficulties, it is not possible in any single case to produce both triploid and tetraploid hybrids between the same species. The quantitative relationship between the genomes included in the various combinations is consequently entirely decisive for the result of the cross-pollinations in such species combinations and cross directions as are at all compatible, just as the quantitative relationships were determining in the intraspecific cross experiments as well. The compatibility between the species in general depends, however, on the con-

stitution of the components taking part in the crosses; and, thus, the chromosome number of the various, possible species hybrids as well as the embryonic environment capable of producing them is determined by a combination of qualitative and quantitative factors. Most likely, the qualitative factors are mainly to be found in the genotype, but plasmatic factors are also possible.

There is a close parallel to the relationships between diploid and tetraploid *L. purpureum* and *L. amplexicaule* in the relations between the diploid and tetraploid forms of *Lycopersicon pimpinellifolium* ($2n = 24$) and *L. peruvianum* ($2n = 24$), elucidated by the well-known embryological investigations by COOPER and BRINK (1945). The species can not hybridize when diploid, nor can diploid and tetraploid forms of *L. pimpinellifolium* do so. On the other hand, tetraploid *L. pimpinellifolium* can hybridize with *L. peruvianum*. The authors cannot offer an explanation; on the contrary, they point out that cases like this prove the impossibility of predicting the crossability between species by means of analogisms.

The compatibility conditions pertaining to *L. hybrido-amplexicaule* and *L. purpureo-amplexicaule* present further striking examples of the unaccountability as far as the crossability is concerned. Besides being compatible with tetraploid *L. amplexicaule*, the former allopolyploid does also hybridize with *L. intermedium*, which — as previously mentioned — is not compatible with *L. hybridum* proper. On the other hand, *L. purpureo-amplexicaule* is neither compatible with tetraploid *L. amplexicaule*, *L. hybridum* nor even *L. intermedium*, in spite of the latter being an allopolyploid between *L. purpureum* and *L. amplexicaule*. A slight swelling of the ovaries of the three combinations shows that fertilization has taken place. In the cases just mentioned, the qualities of the different species and allohexaploids obviously play a much more important part for the compatibility of the various combinations than the degree of relationship between the components.

Above has been pointed out the eventual influence of the choice of the parent lines on the compatibility between the species involved. The results of tetraploid *L. purpureum* × diploid *L. amplexicaule* are particularly illustrative. The lines used no doubt belong to the pure species (*cf.* BERNSTRÖM, 1952, and in press; *p*7 is the tetraploid line of *p*2, *p*42 is that of *p*26, and *p*43 that of *p*29), and consequently the compatibility may to a high degree depend not upon the bulk of genes of the species but on particular genes or groups of genes; however, the

plasmons may also play a part in this connection. Since only a few lines have been used for crosses between tetraploid *L. purpureum* and diploid *L. amplexicaule*, it is quite probable that still greater differences may exist between the lines as far as their crossability is concerned. This is of interest in view of *L. intermedium* being an allopolyploid between *L. purpureum* and *L. amplexicaule* which are incompatible as diploids. It would have been interesting to investigate if those lines which are most pronouncedly incompatible when the *L. purpureum* line is tetraploid, may possibly be compatible when both components are diploid.

SUMMARY.

By doubling the chromosome numbers of the diploid species *L. purpureum* and *L. amplexicaule* it has been possible to produce, with the allotetraploid species *L. intermedium* and *L. hybridum*, hybrids in four new species combinations as against only one previously. Of the various hybrids three are tetraploid and two are triploid. It is not possible to produce hybrids with but a definite chromosome number in any single species combination. Qualitative factors determine the compatibility between the species as much as quantitative ones, *i. e.* the chromosome numbers of the parents, and there is hardly any connection between the degree of compatibility and the degree of relationship. Examples are given of varying compatibility within one and the same hybrid combination, depending on the choice of the male as well as of the female partner. Autotriploids of *L. purpureum* and *L. amplexicaule*, respectively, can not be produced, in contrast to the tetraploid *L. hybridum* where an autohexaploid plant has been obtained. Two artificial allohexaploids were included in the cross experiments.

The most common cause of incompatibility is probably to be found in an inability on the part of the endosperm to develop in the normal way, and in starvation of the embryos. The development of endosperms and embryos proceeds to a different degree in various incompatible cross combinations. The development of hybrid seeds is far better in summer than in autumn.

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AUTOTETRAPLOID SUGAR BEETS

VITALITY CHANGES IN SUBSEQUENT GENERATIONS

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INTRODUCTION.

IMMEDIATELY after the introduction of the colchicine method experiments were started in order to find methods of producing tetraploid sugar beets by colchicine treatment. Those experiments were soon successful and a production of tetraploids from varying diploid types was started. The c_1-tetraploids were expected to be low in vitality and productivity and there is no doubt but that this expectation was fulfilled. The number of plants of the c_1-generation was, however, always very small and the c_1-seeds were not comparable to the normal diploid seeds. Therefore no attemps were made to carry out any field trials in order to determine how inferior the c_1-tetraploids were. In several cases the c_2-seeds could be grown under somewhat normal conditions and the c_3-seeds were usually produced under normal conditions and in lots big enough to allow testing of the tetraploid types in normal field trials.

The experience of polyploids was at that time that the doubling of the chromosome number of plants with low numbers very often produced a considerable increase in the vegetative parts. That experience made it fairly safe to predict that the tetraploid sugar beets would surpass the diploids at least in root size and probably also in sugar production. The first field trials which could be made with tetraploids indicated, however, that the tetraploids also in c_2 and c_3 were very much inferior to the diploids in vitality and productivity. They were in fact, as will be shown below, so much inferior to the diploid breeding material that they would never have been used even for an attempt at breeding, had they been anything else than the in themselves interesting tetraploid sugar beets.

Because the behaviour of the tetraploids deviated from the expectation it was desirable to look for causes to that behaviour other than the tetraploidy *per se*. One such cause could be found in the very narrow genealogical basis of most of the tetraploid progenies — each

tetraploid type was usually derived only from two c_0-plants and the c_1 of each progeny usually was made up of only 5—10 plants. That could be expected to produce rather marked (c_1 = sister plants and c_2 derived from a narrow sibcrossing) inbreeding effects in lowering vitality and productivity. The accumulated experience from numerous inbreeding experiments made it probable that the narrow basis of the tetraploid types might partly explain their inferiority — but only partly. By inbreeding to the degree used in producing the c_3-generation of tetraploids only about half the deterioration in productivity shown should have been expected.

Another explanation to the inferiority of those first tetraploid generations might be that they contained numerous individuals which were cytologically and genically unbalanced and therefore lacking in vitality. If that was the main cause of their inferiority the tetraploids might be expected in some later generations to improve when their genomes got balanced. Only continued growing of successive generations could tell whether this would happen or not. In order to investigate this hypothesis a series of experiments were planned and, partly, carried out.

A further incentive to carry on working with the tetraploids was the hope that the doubling of the number of genes would make selection in the tetraploids more effective than in the diploids and thus enable the breeder to produce tetraploid types surpassing the best diploids in vitality and productivity. Considering that the interaction of the genes rather than their number determines vitality and quantitative characters in general this, however, could at that time be considered just a hope with no very strong foundation neither in theory nor in experience. Special experiments are needed to give an answer to this question. Such experiments are also under way and their result will be reported in due time.

This paper will mainly deal with the question whether the tetraploids automatically improve their originally low standard of vitality by arriving at genical balance in generations later than c_3.

It is desirable not only to know how any single tetraploid progeny behaves but to arrive at a more general knowledge about how tetraploids as such behave. Thus it has been necessary to investigate the behaviour of so many tetraploid progenies that their bulk to some extent can be considered as representative for tetraploids in general. Therefore the material presented comprises as many progenies as it has been possible to test. Economics, however, set a limit for what can be done in the line of such investigations and therefore the number of

progenies tested is more limited than would be desirable in order to make possible generalizing conclusions.

Still another point of importance must be briefly discussed. Vitality and productivity must always be defined as vitality and productivity *under specified conditions of living.* That makes genic adaptation to the conditions the basis of vitality. The tetraploids discussed here have all been derived from diploid material adapted to Scanian beet growing conditions and the tests comparing diploids and tetraploids reported below have been carried out mainly under those conditions. Expecting the tetraploids to inherit the adaptation of their diploid parents, that was expected to make the comparisons less varying. Experience, however, has shown that there was not much gained by this precaution, because the tetraploids in general show reactions towards weather conditions, clearly deviating from those of the diploids. Thus the same tetraploid seed lot may in one year, when the weather is favourable for the tetraploids, give much better results in relation to the diploids than in another year, when the weather may be less favourable for them. This, of course, causes some difficulties for the interpretation of the experimental results, because it usually has not been possible to extend the testing of different generations of tetraploids over the three or four years needed.

MATERIAL AND METHODS.

All the tetraploid material described below has been produced by dipping young flower stalks in colchicine solution (J. RASMUSSON and A. LEVAN: Tetraploid sugar beets from colchicine treatments. Hereditas XXI, 1939, p. 98). The colchicine treatment and the subsequent searching for tetraploids and checking of the tetraploid progenies and their seed producing plants have been made by Dr. A. LEVAN, now of the University of Lund, and Dr. G. VON ROSEN at the Hilleshög Institute.

The commercial seed of the Hilleshög sugar beet variety is a bulk cross of some 20 strains consisting of about 95 % F_1 plants between any two of the fundamental strains. Each of those strains were at the time, when the tetraploid material treated here was created, although genically heterogeneous, very stable and to some extent inbred by mother-family breeding. They were kept stable by continued mass selection under as nearly as possible unchanged cultural conditions.

The material used in this investigation is derived either from the commercial Hilleshög seed or from the mother strains of that seed. The list of tetraploid groups tested is given in Table 1.

TABLE 1. *Tetraploids tested.*

Name	Number of c_0 mother plants	Colchicine treatment in the year	Origin and selection
4*n* Hh	2	1938	Commercial Hilleshög seed. From c_3 split up into several groups
4*n* G+J	2	»	F_2 between strains G and J. From c_3 split up into several groups.
4*n* C	3	1941	Diploid strain C. No selection
4*n* D	7	»	» » D. » »
4*n* G	7	»	» » G. c_4 selected
4*n* H	8	»	» » H. No selection
4*n* I	4	»	» » I. c_4 selected
4*n* J	4	»	» » J. No selection
4*n* Q	4	»	» » Q. c_4 c_5 selected
4*n* U	5	»	» » U. c_4 »
4*n* Y	2	»	» » Y. c_4 c_5 »
4*n* Ä	2	»	» » Ä. » » »
4*n* Sv	3	»	» » Sv. c_4 »

The first tetraploids, 4*n* Hh and 4*n* G + J, were produced when the technique for their production was not very well developed and came into being more or less as luck would have it. The tetraploid strains C to Sv were the results of a determined effort, when the technique had improved. It was decided as far as possible to avoid too low numbers of c_0 parents and only to bring into this investigation strains, the c_1 of which had at least 15 plants — all in order to decrease the difficulty of having the results obscured by strong inbreeding effects. Those demands on the material, however, cut down the number of strains from desired 20 to 11.

The selection mentioned in the last column of Table 1 means a mass selection with balanced consideration given to important quantity and quality characters (root size, root shape, sugar content, ash content, etc.). 1—2 % of the roots grown for selection were selected for propagating the strains. »c_4 selected» should in fact have been »c_4 produced from selected c_3 roots» because the generations labelled »selected» are in fact those derived from selected plants of the preceding generation.

TESTING METHODS.

Vitality and productivity being the characters to be considered make the testing of the material a very important part of the investigation. There are usually four values given for judging the tested material,

i. e. »Dt sugar/ha», »Sugar content», »Dt roots/ha» and »Number of plants/ha». (Dt=deciton=100 kgs. ha=hectare.)

»Dt sugar/ha» is computed from »Dt roots/ha»×»Sugar content» and is obviously the most important measurement of the productivity.

»Sugar content», expressed as % of fresh beet weight, has been determined by hot water digestion and precipitating by lead acetic, filtering and reading the sugar concentration in a common sugar factory polarimeter. The pulp for the digestion has been made by machine as a random sample from all roots of a test plot.

»Dt roots/ha»: All beets from each tested plot were brought into the laboratory, washed by machine and weighed. The plot size being known with a high degree of precision, the root weight from a plot or a number of plots permitted the calculation of the root yield per hectare.

»Number of plants/ha» is expressed in 1000 of plants. The field experiments have been grown in the manner used by 95 % of Swedish beet growers, *i. e.* 45 cms between drill-rows and singled to 25 cms between plants in the row. Theoretically that should give 88000 plants pro hectare but unavoidable disappearance of plants normally makes 70000 to 80000 the actual number of plants and deviations of 2000—3000 plants from the average in an experiment do not affect the yield very much — a few gaps being compensated by more vigorous growth of the plants surrounding the gaps.

It is possible to estimate the yield of a full plant population from a gappy one but the calculations are extremely arbitrary. Therefore no attempts have been made to calculate the theoretical yield. Since plant number usually is a function of vitality this gives a further cause for not trying to calculate any yield figures by compensating for deficient plant populations. A few experiments which would not be of great importance and where the plant number has been very deficient have been left out of the report.

The field experiments have normally been laid out as randomized replicated plots with plot size 2 rows 0,45 m apart and 12 m long. From time to time the number of replications within an experiment has varied from 4 to 10. The report has been confined to the results of experiments laid out in Scania and most of the field trials have been carried out in the neighbourhood of the Institute, *i. e.* at the Hilleshög or the Säbyholm estate. The extent of the field experimentation is in the tables given as number of plots tested — that giving the best information about the validity of the figures.

It will mostly be found that the sugar yields presented in the tables will not arithmetically fully agree with the product of root yield and sugar content. That is due to the fact that the sugar yield has been calculated for each plot separately and the sugar yield in the tables being the average of the plot-yields of sugar.

The directly found figures from the experiments tell nothing about the characters of the tested types because they vary with soil and weather. Therefore the results are expressed as comparisons between the tested tetraploids and a standard diploid material which has been grown in all experiments. As standard has mostly the Hilleshög K variety been used. That one is somewhat different (5 % higher yield, 0,3 lower sugar content) from the Hilleshög variety from which most of the tetraploid material under discussion has been derived. It has, however, the advantage of being very well adapted to the Scanian beet growing conditions as well as being highly physiologically adaptable to varying conditions. Genically it is known to be reasonably stable which is shown by the fact that seed lots produced in different years give practically the same results when tested side by side.

The bulk of the figures of the investigation are given as difference of tested type minus standard.

On comparing the tetraploids to the diploid standard a certain amount of undesired variation in the results is caused by the fact, mentioned in the introduction, that the tetraploids in general react towards weather conditions in another way than the diploids. This is, however, the lesser evil and cannot be avoided.

TEST RESULTS.

SEED PRODUCTION.

It might be expected that the early generations of new tetraploids would have difficulties in their meiosis causing partial sterility and low yield of viable seeds. It might equally be expected that those difficulties would be overcome in some later generations resulting in a somewhat normal yield of viable seeds.

No special experiments have been carried out in order to verify whether changes in the seed yield take place. An attempt has been made to find out whether the results from the seed growing indicate any improvement in the seed yield as the tetraploids grow older. The influence on the seed yield of the soil together with the necessity to grow the tetraploid seed in a distance several kilometers apart from

the diploid makes all comparison impossible and thus there is no information available about the inherent seed production of different tetraploid generations.

LEAF COLOUR.

The leaf colour of the first tetraploids was very much lighter than that of the comparable diploids. The difference held for a couple of generations but began to disappear in the c_3—c_4 generations. Today the old tetraploids have very nearly the same colour as the diploids. In the colour characteristic there is thus a definite change in the tetraploids but available notes do not allow fixing the stage where the change has taken place.

YIELD INVESTIGATIONS.

The first time it was possible to compare the yield of tetraploid and diploid sugar beets was in 1939 when a group of c_2 families of 4*n* Hh were compared to a bulk cross of 4 diploid strains. There was not sufficient seed available to lay out a proper field trial. Each family was grown on one drill-row of 10 m length and every second row was sown with diploids. Only three of the 4*n* families gave enough plants to allow a comparison and even those families had more gaps than the 2*n* rows. Each beet was weighed and analysed separately and the result of the test is best presented by comparing the plant averages. Because of the gaps in the 4*n* rows the tetraploids must be deemed to be favoured by having bigger roots than they would have had in a complete stand. The comparison comprises 50—60 plants of each type. Table 2 shows the average plant values found.

TABLE 2. *c_2-tetraploid families of 4n Hh compared to 2n bulk cross plants in 1939.*

	Root weight, grams	Sugar content %	Grams sugar pro plant
2*n*	262	15,0	39
4*n*	193	15,5	31
2*n*—4*n*	+ 69	— 0,5	+ 8

Another set of c_2-families of 4*n* Hh were tested in 1940 and compared to the same 2*n* bulk cross as was used in the 1939 test. Each family was sown on a 10 m long row and groups of five 2*n* and five 4*n* rows alternated. The whole harvest from each row was weighed and

analysed. Nearly half of the 4*n* rows gave very deficient plant populations and have had to be excluded from Table 3 where the results are presented.

TABLE 3. *Diploids and c_2-tetraploid families of 4n Hh compared on 5,4 m^2 plots 1940.*

	Yield of roots Kgs pr plot	Sugar content %	Yield of sugar Kgs pr plot
2*n*	17,3	18,1	3,15
4*n*	16,0	18,0	2,83
2*n*—4*n*	+ 1,3	+ 0,1	0,32

The results from 1939 and 1940 have been presented because they demonstrate the first impressions given by the first tetraploid sugar beets. The impression of their inferiority was still stronger than that indicated by the figures because in those experiments all possible care had been taken to give all the plants optimal germination and growing conditions. That made those who worked with them suspect that the tetraploids under normal conditions would do considerably worse.

In 1941 it was possible to start a series of properly arranged field trials with c_2 of 4*n* Hh and in 1942 with c_3 of 4*n* Hh and 4*n* G+J. Those tests have since then been carried on to the c_6-generation of both tetraploid types. The results are presented in Table 4.

The tetraploid types are derived from two c_0-plants each and thus we may expect some depression caused by the inbreeding from sister plants, although it is impossible to know what part the inbreeding plays in producing the inferiority of the tetraploids under the diploids. The two tetraploids behave very much alike which might be expected because the diploid Hh and the diploid G+J descendants are very much alike.

The striking thing about Table 4 is that both tetraploids in the c_6-generation suddenly give much better yield results than they have done in the preceding generations. The c_6-generation of both these tetraploids may be considered equal to any diploid derived from a two-plant family. There also seems to be a step upwards between the c_3- and c_4-generations.

The interpretation of these results is made somewhat difficult because some selection has taken place in the manner that some of the families or groups brought into the test have been discontinued. Also some selection within the families has been done. Still a thorough survey of the lines of descent gives the impression that selection has

TABLE 4. *Field trials with successive generations of tetraploids 4n Hh and 4n G+ J.*

Figures given are tetraploid minus Hilleshög K.

Tetraploid generation	Test years		Total replications		Dt sugar/ha		Sugar content		Dt roots/ha		Number of plants/ha	
	4n Hh	4n G+J	4n Hh	4n G+J	4n Hh	4n G+J	4n Hh	4n G+J	4n Hh	4n G+J	4n Hh	4n G+J
c_2	1941	—	18	—	−11,4	—	0	—	−64	—	−16	—
c_3	1942	1942	18	18	−13,8	−13,7	+0,5	+0,5	−84	−85	− 5	−5
c_4	1944 1945 1949	1944	56	16	−10,0	− 9,6	−0,4	+0,3	−50	−55	− 5	−6
c_5	1946	1946	20	24	−10,4	−11,0	−0,2	+0,4	−49	−59	− 2	−7
c_6	1950	1950	4	4	− 4,0	− 1,0	−0,7	+0,2	− 5	−11	+ 2	−1

TABLE 5. *Tests with c_2, c_3 and c_5 of tetraploid strains 4n H, 4n Q and 4n Y.*

Figures represent strain minus Hilleshög K.

Tetraploid generation	Test years			Total replications			Dt sugar/ha				Sugar content				Dt roots/ha				Number of plants/ha			
	4n H	4n Q	4n Y	4n H	4n Q	4n Y	4n H	4n Q	4n Y	Mean	4n H	4n Q	4n Y	Mean	4n H	4n Q	4n Y	Mean	4n H	4n Q	4n Y	Mean
c_2	1945	1945	1942	8	8	9	−1,5	−22,0	−18,4	−14,0	+0,5	+0,9	+0,1	+0,5	−15	−130	−109	−85	−6	−6	−5	−6
c_3	1946 1947	1946 1947	1946 1947	24	24	24	−5,2	−17,0	−14,9	−12,4	+0,5	+0,1	−0,6	±0,0	−38	− 99	− 70	−69	−1	−4	−2	−2
c_5	1951	1951	1951	10	10	10	+3,1	− 3,3	+ 2,2	+ 0,7	+0,6	+0,6	−0,2	+0,3	− 6	− 67	+ 16	−19	+3	−5	−1	−1

played a very minor rôle in producing the improvement. Therefore the results presented in Table 4 are best interpreted as indicating that an automatic improvement has taken place in the two artificial tetraploids in question.

As mentioned above, when tetraploids of the strains 4*n* C to 4*n* Sv were produced, care was taken to build the new tetraploids on a broader basis than that used for 4*n* Hh and 4*n* G+J. With the exception for C, Y, Ä and Sv the number of c_0-plants makes it probable that no very marked inbreeding effect may be encountered. The comparatively great number of c_1-plants also helps to avoid close inbreeding. The worst disturbance in this series of tetraploids is that the c_3-plants due to adverse conditions produced very little and poor seed. Therefore no tests could be carried out with the c_4-generation, all seed being needed for propagation of the strains.

Three of the strains H, Q and Y were tested in c_2, c_3 and c_5. The results of these tests are presented in Table 5. Although there is a variation between the strains, which might have been expected, they agree in the trend of the changes between generations, *i.e.* c_3 ist slightly better than c_2 and c_5 is much better than c_3. There is, however, one difficulty for the interpretation, namely the fact that the year 1951, when c_5 was tested, was very favourable to the tetraploids in general. That makes the c_5 appear better than it actually is. From trials with the same seed lots in 1950 and 1951 it may be concluded that the 1951's favouring of the tetraploids at the most amounts to 3 dt sugar pro ha and about 20 dt of beet. Even when subtracting these values from those for c_5 in Table 5 the c_5-generation shows a very marked improvement as compared to the c_3-generation. These results, thus, agree with those of the 4*n* Hh and 4*n* G+J in giving a strong indication that the artificial tetraploids start as very inferior types but automatically improve in later generations.

Table 6 shows the comparisons between c_3 and c_5 of all the strains 4*n* C—4*n* Sv. That material has been divided up in Tables 6 *a*, giving the results from the not selected strains, and 6 *b* giving the results of those strains which have been submitted to selection between c_3 and c_5.

This series as a whole is practically undisturbed by annoying deficiency in plant numbers and we may safely conclude that the plant numbers (which may be caused by different viability of young plants) have had no influence on the yield differences.

The over all averages of Table 6 show a very marked improvement between c_3 and c_5 and the improvement is in all characters greater in

TABLE 6. *Comparison between c_3 and c_5 of strains 4n C to 4n Sv.*

Figures represent strain minus Hilleshög K. Test years: c_3 1946 and 1947, c_5 1951. Replications: c_3 24, c_5 10.

Strain	Dt sugar/ha		Sugar content		Dt roots/ha		Number of plants/ha	
	c_3	c_5	c_3	c_5	c_3	c_5	c_3	c_5
6a. Not selected strains								
4n C	— 13,2	— 3,2	+ 0,2	+ 1,1	— 72	— 37	+ 5	+ 2
4n D	— 9,8	— 1,2	± 0,0	+ 0,1	— 52	— 8	— 2	± 0
4n H	— 5,2	+ 3,1	+ 0,5	+ 0,6	— 38	— 6	— 1	+ 3
4n J	— 4,0	+ 4,6	+ 0,1	+ 0,3	— 24	+ 19	— 2	— 4
Average 6a	— 8,1	+ 0,8	+ 0,2	+ 0,5	— 47	— 8	± 0	± 0
6b. Strains selected between c_3 and c_5								
4n G	— 4,4	+ 2,5	— 0,2	+ 0,9	— 19	— 4	+ 2	± 0
4n I	— 7,3	+ 1,1	+ 0,5	+ 1,2	— 53	— 10	± 0	— 2
4n Q	— 17,0	— 3,3	+ 0,1	+ 0,6	— 99	— 67	— 4	— 5
4n U	— 21,3	+ 3,3	— 0,9	— 0,8	— 76	+ 34	— 11	— 2
4n Y	— 14,9	+ 2,2	— 0,6	— 0,2	— 70	+ 16	— 2	— 1
4n Ä	— 3,5	+ 5,1	— 0,5	+ 0,1	— 9	+ 27	— 5	+ 2
4n Sv	— 7,4	— 2,4	— 0,4	± 0,0	— 32	— 14	+ 5	± 0
Average 6b	— 10,8	+ 1,2	— 0,3	+ 0,3	— 51	— 3	— 2	— 1
All over average	— 9,8	+ 1,1	— 0,1	+ 0,4	— 49	— 5	— 1	— 1

the selected group of strains than in the 6*a*-group. None of the differences 6*b*—6*a* surpasses the P-level of 0,2. Although there is an indication that the selection has some part in the improvement of the 6*b*-group the selection effect is obviously so small that it may be neglected and the whole set of strains can still in c_5 be considered as a uniform group. The changes between c_3 and c_5 may, therefore, be characterized as follows.

	Diff. c_5—c_3	±m	T	P
Beet yield	+ 44	3,1	14,3	< 0,001
Sugar content	+ 0,3	0,12	2,5	0,05—0,01
Sugar yield	+ 10,9	1,14	9,5	< 0,001

Even if a favourizing of the c_5 by the 1951 testing is taken into account it is obvious that a very considerable and statistically highly significant improvement of the strains automatically has taken place between c_3 and c_5.

Since the inferiority of the newly produced tetraploids is amply

demonstrated it may be well to fill out the picture by mentioning that the hybridization effect in the tetraploid material is considerably greater than in the diploid material and also that from early tetraploid bulk crosses new tetraploid strains have been selected which all give a very good promise. Some of them are even tested so far that their superiority over all diploid strains and even over the best available diploid bulk crosses may be considered as firmly established. (c_6 and c_7 tested in 1950, 1951 and 1952, totally 27 replications giving 24 dt of beets, 0,28 % sugar content and 5 dt of sugar pro ha above the best available diploid bulk cross.)

DISCUSSION.

The evidence of the investigations presented above is as consistent as may be expected from a material of the kind used for the experiments. It all goes to show that the newly produced artificial sugar beet tetraploids are very much inferior in vitality and productivity to the diploids. It also shows, however, that the tetraploids without human selection improve and that a big step in the improvement is taken between c_3 and c_5 (in some cases between c_5 and c_6).

All the tetraploids tested in this investigation being produced by one tetraploidizing method the conclusions must be confined to tetraploids produced by that method. It is thus an open question whether tetraploids produced by other methods will behave in the same manner as those discussed here.

The material tested is of course too small to allow generalizing the results as being valid for tetraploid sugar beets as such. In lack of other evidence it still seems probable that tetraploid sugar beets produced by the flower stalk dipping method have low vitality and productivity at least in the c_1- to c_2-generations and that their vitality and productivity in the following generations without intentional selection rise to a level equal to the normal level of comparable diploids.

The low vitality of the first tetraploid generations may be thought to be caused by disturbed chromosomal and gene balance in the newly formed autotetraploids. In consequence, their improving in later generations may be thought to be the result of a natural selection process eliminating the unbalanced types of gametes, embryos and seedling plants. It may also be mentioned that a few experiments (not reported here) where c_2-tetraploid strains have been crossed have produced a c_3-F_1 which immediately rose nearly to the level of the diploids and in later generations did not materially improve by itself.

The results reported here tend to show that c_1, c_2 and c_3 usually are fairly alike and that the improvement appears rather suddenly in one or two later generations. That does not agree very well with the general conception of how a natural selection works. The phenomenon may, however, find an explanation in the fact that the c_1- and c_2-generations with few plants and known to be weak usually are handled with extreme care in order to save every plant for the production of sufficient c_3-seed. Whether this explanation is sufficient or whether another must be sought must at the moment be left an open question.

SUMMARY.

Subsequent generations (c_2—c_6) of colchicine-produced sugar beet strains have been tested in field trials and their yields of beets and sugar have been compared by means of their deviations from a diploid standard type. The difference in physiological reactions between diploids and tetraploids causes some uncertainty in the interpretation of the results. Still it has been demonstrated that the tetraploids between the c_3- and c_6-generations automatically improve their, originally low, productivity so much that they at least reach the level of comparable diploids.

All the tetraploids tested being produced by the flower stalk dipping method the conclusions must be confined to tetraploids produced by that method. With that reservation it seems probable that the observed behaviour of the tested material is a common occurrence in artificial tetraploid sugar beets.

DIFFERENTIAL RESPONSE TO MUTAGENS AS STUDIED BY THE NEUROSPORA REVERSE MUTATION TEST

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IN a previous paper KÖLMARK and WESTERGAARD (1953) compared the mutagenic effect of different chemicals on the induction of back-mutations in the purple adenineless strain of *Neurospora crassa*.

On the basis of these results it became of considerable interest to test the same compounds also on some other biochemical mutants. This became especially important after the discovery of the very strong mutagenic effect of some epoxides. It was thought that by extending the back-mutation test to other loci it should be possible to reveal a specificity in the sense that certain mutagens might be especially effective on certain loci.

A selective effect of this kind would, of course, have interesting theoretical and practical implications.

For such an extension of the back-mutation test the employment of mutant strains the reversion patterns of which are known is desirable. A number of such strains has been put at our disposal by NORMAN H. GILES JR. of Yale University, and these strains were employed in the course of the present investigations.

GILES (1951; *cf.* particularly Table 12) studied the spontaneous and ultraviolet light (u. v.) induced reversion patterns of eight different inositol dependent mutants of *Neurospora crassa*. Although the strains seemed to be physiologically and genetically identical (in one case, however, genetical evidence indicates a compound locus; GILES, 1951, p. 307) it was shown that they exhibited marked differences in their spontaneous and u. v. induced reversion pattern. GILES was able to classify the mutants into five different groups, designated groups I—V. Group I, comprising one mutant, originally produced by nitrogen mustard, was characterized by a very high spontaneous reversion rate, which was not enhanced by u. v. treatment. Group II, likewise represented only by one mutant, which had been induced by u. v., showed a low spontaneous reversion rate, but a very high response to u. v. irradiation. Group III, which was represented by four mutants, induced

by u. v. and nitrogen mustard, likewise had a low spontaneous reversion rate, but the response to u. v. was approximately one third of that of group II. Group IV, again, comprising one nitrogen mustard induced mutant, had a very low spontaneous reversion rate, which could be only very slightly enhanced by u. v. treatment, whereas it responded somewhat better to X-ray treatment. Group V, represented by one u. v. induced mutant, was never found to revert, either spontaneously or after treatment with mutagens; there is good evidence that one deals here with a chromosome mutation.

Preliminary genetical evidence revealed that the reversions from inositol dependence to inositol independence could occur either through back-mutations or by suppressor mutations.

The five groups of mutants may be considered as a series of pseudoalleles occupying the same locus on the chromosome, controlling the same biochemical reaction (as far as biochemical evidence goes), and differing only in their spontaneous and induced reversion patterns.

GILES (*l. c.*, Table 17) also made a comparison between the relative frequencies of u. v. and X-ray induced reverse-mutations in group II and group IV. U.-v. induced 3,6 times as many mutations in group II as X-rays. In group IV, X-rays produced 25 times as many mutations as u. v.

Our material also includes a methionine requiring mutant, likewise studied by and provided by Dr. GILES. This mutant, in which the biochemical step controlling the coupling of cysteine and homoserine to cystathionine is blocked, is unable to revert by back-mutations, but does revert both spontaneously and after u. v. treatment by suppressor mutations. GILES (*l. c.*) was able to demonstrate the existence of two non-linked suppressors, one of which proved to be closely linked to albino-2.

EXPERIMENTAL RESULTS.

One inositolless strain of each of GILES' five groups, namely: 5202 of group I, 37401 of group II, 37102 of group III, 89601 of group IV, and 46802 of group V was crossed with the extreme colonial type, which is very suitable for plating, and which has been designated as »distinctus» (KÖLMARK and WESTERGAARD, 1953). The methionineless strain was also crossed with the »distinctus» strain. The biochemical mutants in the »distinctus» colonial form were then obtained by single ascospore isolation. The general procedure for treatment with mutagens and for scoring the reverted colonies is the same as that reported previously (JENSEN *et al.*, 1951; KÖLMARK and WESTERGAARD, 1953).

TABLE 1. *Mutants: Inositolless, groups I—V. — Treatment: ultraviolet light, 60 sec. and 0,06 M H_2O_2 + 0,03 M HCHO, 25 min. — Ca. 200 × 10^6 conidia treated in each experiment.*

Gene	Treatment	Per cent survived	Mutations		
			Number counted	Per 10^6 initial	Per 10^6 survived
inos-	control	100	1560	8,1	8,1
group I	u. v.	75	2034	10,6	14,0
	H_2O_2+HCHO	40	1584	8,3	20,5
inos-	control	100	3	0,03	0,03
group II	u. v.	71	1204	10,4	14,7
	H_2O_2+HCHO	38	5	0,04	0,12
inos-	control	100	3	0,01	0,01
group III	u. v.	71	366	1,6	2,2
	H_2O_2+HCHO	44	51	0,2	0,5
inos-	control	100	1	0,004	0,004
group IV	u. v.	85	5	0,02	0,03
	H_2O_2+HCHO	30	11	0,05	0,16
inos-	control	100	0	—	—
group V	u. v.	1	0	—	—
	H_2O_2+HCHO	(+)	0	—	—

The first mutation experiment was designed to test whether the biochemical mutants in the new colonial form would give the same spontaneous and u. v. induced reverse mutation rates as those previously found by GILES (*l. c.*). The results are given in Table 1. It appears that the spontaneous as well as the u. v. induced mutation rates obtained in all five groups are in good accordance with the data given by GILES.

In the course of the same experiment the strains were also treated with a mixture of hydrogen peroxide and formaldehyde, a treatment which is known to have a good effect on the reversion rate of the adenine locus (KÖLMARK and WESTERGAARD, 1953). The chemical treatment is more effective in group III than in group II, while in the case of u. v. treatment the reverse is true. It will also be noted that u. v. treatment is more effective than this chemical treatment in both these groups. In group IV a few more mutations are found following chemical as compared with u. v. treatment. The figures, however, are so low that their significance must await further confirmation. Group V showed no mutants. The strains belonging to this group frequently show poor growth even on the complete medium. This may be caused by chromosomal disturbances, which may also account for the low survival rate found during this experiment.

TABLE 2. *Mutants: Inositolless, groups I—IV (inos⁻). — Methionineless (meth⁻). — Adenineless (ad⁻). — Treatment: 0,2 M 1 : 2,3 : 4-diepoxybutane.*

Gene	Treatment	Per cent survived	Number counted	Mutations Per 10^6 initial	Mutations Per 10^6 survived
inos⁻	control	100	10000	74,7	74,7
group I	diep. 24 min.	46	8140	60,7	128,0
inos⁻	control	100	0	—	—
group II	diep. 24 »	49	2	0,02	0,02
	» 36 »	4	26	0,23	6,3
inos⁻	control	100	0	—	—
group III	diep. 24 »	54	58	0,5	0,8
	» 36 »	14	76	0,6	4,1
inos⁻	control	100	0	—	—
group IV	diep. 24 »	43	4	0,02	0,04
meth⁻	control	100	204	2,8	2,8
	diep. 24 »	46	386	5,3	11,6
	» 36 »	4	182	2,5	70,0
ad⁻	control	100	0	—	—
	diep. 24 »	62	4540	22,3	36,2

In the next experiment the mutants of groups I—IV were exposed to treatment with 1 : 2,3 : 4-diepoxybutane, the mutagen which had shown a very strong effect on the adenineless strain (KÖLMARK and WESTERGAARD, 1953). The methionineless strain described above, as well as our adenineless strain, were treated in the same manner. Results are recorded in Table 2. In all the inositol-strains a small but noticeable effect is found. Again, the effect of the chemical is slightly better in group III than in group II. In the methionineless strain, a somewhat better effect has been found, but unfortunately this strain also showed an unusually high »background» in spontaneous reverse mutation rate during this experiment. The chemical had a very striking effect on the adenine locus. The number of mutations rises here from zero in the control to 4500 after 24 minutes of treatment.

Now the question arises: Does the different response of the inositolless and the adenineless genes to the same treatment reflect inherent differences between the genes, or is it due to secondary differences, *e. g.*, differences in the permeability of the cells of the strains used, *etc.*? In order to obviate these secondary differences a double mutant was

TABLE 3. *Mutant: Double mutant, adenineless, inositolless (group II). — Treatment: ultraviolet light, 60 sec. — 0,2 M 1:2,3:4-diepoxybutane.*

Treatment	Per cent survived	Number counted		Mutations Per 10^6 initial		Per 10^6 survived	
		ad +	inos +	ad +	inos +	ad +	inos +
Control	100	4	0	0,04	—	0,04	—
u. v. 30 sec.	50	444	496	3,9	4,4	7,8	8,7
90 »	34	378	578	3,3	5,1	9,2	14,8
150 »	15	200	318	1,8	2,8	11,7	18,7
210 »	5	54	92	0,5	0,8	10,2	17,4
Diepoxybutane							
0,2 M 6 min.	100	780	19	6,8	0,2	6,8	0,2
12 »	83	3320	17	29,2	0,2	34,2	0,2
24 »	47	10130	20	88,8	0,2	191,0	0,4
36 »	9	9300	20	81,6	0,2	894,0	0,2
48 »	0,01	134	0	1,2	—	∞	—

114×10^6 spores in each experiment.

produced in which the adenineless and the inositolless genes are situated in the same nucleus. In this mutant the two genes are under the same physiological and environmental conditions during growth and experiment. In such a double mutant reversions from adenineless to adenine-independence can be scored on minimal medium plus inositol, while reversions from inositolless to inositol-independence can be counted on minimal medium plus adenine. In all the experiments the treated spores were also plated on minimal medium alone, but no double reversions were encountered.

Table 3 shows the results of one such experiment with the double mutant of adenineless and inositolless of group II. It is apparent that the differences between the adenine and the inositol loci in response to mutagenic treatment are maintained in the double mutant. When exposed to u. v. the inositol gene consistently shows a higher reversion rate than the adenine gene. After treatment with diepoxybutane the picture is exactly reversed: Very few reversions are induced in the inositol gene whereas again a very high number of reversions occur in the adenine gene, in conformity with the results obtained with the single mutants. Consequently, the possibility that secondary physiological factors between the two strains were responsible for the differences obtained in response to mutagens can be ruled out. They must thus be manifestations of primary differences between the two loci.

Now the question arises: Are we dealing here with a specific effect of 1 : 2,3 : 4-diepoxybutane, or do the two loci show similar differences

when treated with other chemicals? Preliminary evidence suggests the second possibility. This already might have been anticipated from the data given in Table 1, which showed that treatment with formaldehyde plus hydrogen peroxide, which has a good effect on the adenine locus (KÖLMARK and WESTERGAARD, 1953), has a rather weak effect on the inositolless strains. This conclusion is further supported by data obtained in the course of preliminary and yet unpublished experiments, in which the inositolless strains have been treated with some of the mutagens which have shown positive effects on the adenine locus (mono- and dichloro-mustard, hydrogen peroxide, formaldehyde *etc.*; *cf.* KÖLMARK and WESTERGAARD, 1953). In all instances the mutagenic effect on the inositol locus was low when compared with the effect on the adenine locus, and in all cases, inositolless group III showed a somewhat better response than members of the other groups. It should be added, however, that urethane and phenols, which have no effect on the adenine locus, likewise have been found ineffective in enhancing the reversion rate of any of the inositolless strains. These observations are consistent with those of SMITH and SRB (1951) who have shown that β-propiolactone has a stronger effect on adenine reversions than on reversions in inositolless group II.

None of the above-mentioned chemically induced reversions in the inositolless and methionineless strains have so far been subjected to genetic analysis, and the next step will be to find out whether the reversions induced by chemical mutagens comprise back-mutations as well as suppressors, as was the case with the u. v. induced reversions studied by GILES.

If one pools the information obtained by GILES and us with the inositolless mutants, and that obtained by us with the adenineless mutant and the double mutant, a rather interesting mutation pattern begins to emerge: (1) Two different alleles (pseudoalleles) with the same spontaneous reverse mutation rate may show a different response to u. v. treatment, as exemplified by members of groups II and III in the inositolless series. — (2) The gene with the high response to u. v. treatment may have a low response to X-ray treatment and *vice versa* (see: inositolless groups II and IV; GILES, 1951, Table 17). — (3) A gene with a very high response to u. v. treatment (see: inositolless group II) may have a low response to chemical mutagens. — (4) Chemical treatment has a much stronger effect than u. v. treatment on a different gene, as exemplified by the adenineless locus. The experiments of GILES as well as the experiments involving the double mutant recorded

here have ruled out the possibility that these differences might be due to modifying genes or to secondary physiological differences between the strains. Differences in the mutation pattern thus seem to offer promising material for further studies concerning the mutational processes, and for analysing the mechanisms by which physical and chemical agents can affect the stability of the gene.

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CHROMOSOMAL EVOLUTION IN THE GENUS POLYGONATUM

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THE Liliaceous genus *Polygonatum* has taxonomically been a neglected group. The only paper dealing with the whole genus is BAKER's (1875) revision of the tribe *Asparagaceae*. On the basis of the arrangement of leaves BAKER has divided the genus into three groups: *Alternifolia*, *Verticillata*, and *Oppositifolia*. According to BAKER, the group *Alternifolia* comprises 12 species, the group *Oppositifolia* 7 species, and the group *Verticillata* 4 species.

The genus *Polygonatum* is distributed over the Northern hemisphere. The group *Alternifolia* has the widest distribution, being found in the Old World as well as in the New World. In America this group is solely prevailing. The group *Verticillata*, again, has representatives in Asia and in Europe. Curiously enough, this group is lacking in Japan. The distribution of the group *Oppositifolia* is restricted to the Chinese mountains and Himalaya, the majority of the species being found in the latter region. To this group belongs the southernmost of the Old World species, *Polygonatum kingianum*, which grows in Burma.

The number of *Polygonatum* species known was greatly augmented by an expedition directed to the Southwestern mountains of China and the province of Yun-Nan. The material collected by this expedition has been revised by HUA (1892). He has described 13 new *Polygonatum* species, and his list in which he has included the species, described since BAKER's revision, contains a total of 45 species. Of these, 23 are Chinese.

For the present, some 50 *Polygonatum* species have been described (*cf.* COLLETT and HEMSLEY, 1891; LÉVEILLÉ, 1909, 1913; MAEKAVA, 1932; OWNBEY, 1944). Many of the descriptions are, however, rather uncritical, and no up-to-date revision of the genus *Polygonatum* does exist. In view of the scarcity of the plant material which has been available to taxonomists, and the fact that the chief distribution centre of the genus lies in East Asia, it can be safely assumed that a number of species still await their discoverer.

The present author has carried out cytological studies on the genus *Polygonatum* since 1943 (SUOMALAINEN, 1947; THERMAN, 1950, 1952).

Material has been available only of a part of the described species, East Asiatic species being naturally most difficult to obtain. European and American species have therefore been investigated most extensively. In all, some 100 clones as well as a number of seedlings have been studied cytologically. The present paper is a survey of those cytological facts which have a bearing on the evolution and the taxonomical relationships in the genus *Polygonatum*. It may be mentioned that very few earlier observations on the cytology of the genus do exist, and even those are mostly confined to individual chromosome counts.

I. GROUP ALTERNIFOLIA.

1. OLD WORLD SPECIES.

Of the group *Alternifolia*, three representatives are found in Europe, *viz. Polygonatum officinale*, *P. multiflorum*, and the more Southern *P. latifolium*. The diploid chromosome number of *P. officinale* and *P. latifolium* is 20, while that of *P. multiflorum* is 18. *P. multiflorum* is the only *Polygonatum* species, found so far, to possess this chromosome number. All the other species, belonging to the group *Alternifolia*, exhibit as their haploid number 10 or some multiple of this number.

The morphology of the chromosomes is illustrated by Figs. 1—6. It must be pointed out that the letters do not refer to any possible homologies which may exist between the chromosomes of the different species. The karyotypes in the group *Alternifolia* are of two main types which I have proposed to call the *Officinale*-type (Fig. 2) and the *Latifolium*-type (Fig. 6) (SUOMALAINEN, 1947). The most important characteristic of the former is the presence of a long and a short SAT-chromosome, while the latter type is characterized by the fact that two of the longer chromosomes have secondary constrictions. The *Latifolium*-type has a far wider distribution, most of the Asiatic species and all the American species being representatives of this type.

An analysis of meiosis reveals the homologies of the chromosomes. In all pure species the chromosomes pair regularly in twos; no multivalents or groups of several chromosomes have been observed. The hybrid *P. officinale*×*multiflorum* has, of course, 19 chromosomes, and the parental chromosome types can be discerned in the hybrid (*cf.* Figs. 3—5). In meiosis 16 chromosomes form 8 bivalents, while the remaining three chromosomes form a group of three.

This behaviour is best explained by assuming that 8 of the *officinale*-chromosomes correspond with 8 of the *multiflorum*-chromosomes. The

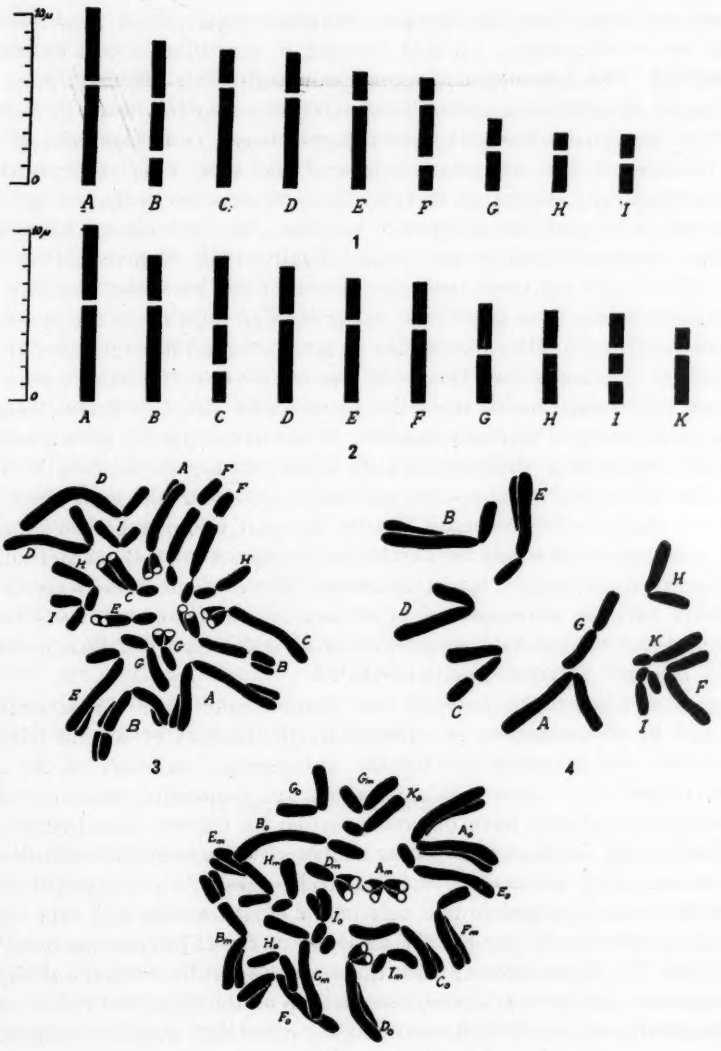


Fig. 1. Idiogram of *P. multiflorum*. — Fig. 2. Idiogram of *P. officinale*. — Fig. 3. Chromosome complement of *P. multiflorum*. — Fig. 4. Haploid chromosome set from a pollen grain of *P. officinale*. — Fig. 5. Chromosome complement of *P. officinale* × *multiflorum*.

three remaining chromosomes have the following structure: *P. officinale* has two chromosomes, 1,2 and 3,4 and *P. multiflorum* one chromosome 2,3. The homologous regions pair with each other, forming a group of three chromosomes. Such a relationship has most probably arisen through an unequal interchange between two chromosomes in a 10-chromosomic set, and the loss of the smaller of the resulting chromosomes. Decrease of the chromosome number, owing to such a process, is of common occurrence in plants, the best-known examples being provided by the genus *Crepis* (TOBGY, 1943; BABCOCK, 1947).

The following facts speak in favour of the view that we also in the present case have a decrease rather than an increase of the chromosome number, in other words that 10 is the original basic number from which 9 is a derivative. The basic number 10 is much more common, being represented by all the other species with alternate leaves, except *P. multiflorum*. The basic number 10 has consequently also a much wider geographical distribution both in the Old and in the New World. In the 10-chromosomic species not one multivalent has been observed which ought to be the result if after unequal interchange the smaller chromosome type would be doubled in number. A process again, leading through interchanges to an increased chromosome complement, in which all the chromosome types are non-homologous, needs more evolutionary steps and coincidences than a decrease (*cf.* DARLINGTON, 1937, p. 559; STEBBINS, 1950, p. 446).

The relationship between the chromosome sets, which contain 9 and 10 chromosomes, is reflected by the meiosis of several triploid hybrids, which possess the somatic chromosome numbers 28, 29, and 30, respectively. These triploids which are frequently encountered in European material have obviously arisen as follows. For instance, a complement of 28 chromosomes contains two sets of 9 chromosomes and one set of 10 chromosomes. Correspondingly, a complement of 29 chromosomes comprises two sets of 10 chromosomes and one set of 9 chromosomes. In meiosis the majority of the chromosomes form trivalents. But in accordance with the diploid hybrid in which a group of three chromosomes is found, the meiosis of the triploid hybrids often reveals a group of four chromosomes or a too high number of bivalents instead of trivalents. In a triploid hybrid *P. officinale*×*multiflorum* with 28 chromosomes, the explanation is naturally the following: 24 chromosomes form trivalents; the remaining four chromosomes have the structure: the *officinale*-chromosomes are 1,2 and 3,4, while two 2,3 chromosomes are inherited from *P. multiflorum*. These chromo-

somes have homologous segments, respectively, and pair to form a group of four.

VESTERGREN (1925) has made a study of the hybrid *P. multiflorum*×*officinale* and its occurrence in Sweden. According to VESTERGREN, the hybrid is more robust and vigorous than either of the parents, being, however, highly variable in its external appearance. In the light of cytological evidence, it seems most probable that the hybrids described by VESTERGREN, actually are triploids. Their variation obviously depends on different strains having different numbers of the parental chromosome sets. VESTERGREN describes also *P. multiflorum* (L.) ALL. var. *bracteatum* (THOM.) KUNTH. This variety, again, obviously is a true diploid hybrid between *P. officinale* and *P. multiflorum*.

An analysis of meiosis reveals that pure species as well as interspecific hybrids are heterozygous in regard to their chromosome structure. In the pure species the most common changes seem to be inversions. The hybrids again exhibit in their meiosis the results of all possible kinds of structural hybridity, both eucentric and dyscentric (*cf.* SUOMALAINEN, 1947).

Changes in chromosome structure have thus obviously been of great importance in the evolution of the group *Alternifolia* in Europe as well as in Asia. Polyploidy seems to be absent in the Old World species of this group, if we disregard the triploid hybrids. The pure species are fertile, whereas the diploid and triploid hybrids are completely sterile. It may be mentioned that in all *Polygonatum* forms vegetative reproduction through rhizomes is very effective.

2. NEW WORLD SPECIES.

In the American branch of the genus *Polygonatum*, which as a whole belongs to the group *Alternifolia*, evolution has taken another course. On this group much more taxonomic investigation has been carried out than on the European and Asiatic species of the genus. A satisfactory classification has, however, proved most difficult to achieve. OWNBEY (1944), in her taxonomic revision of the American *Polygonatum* forms, has extensively summarized the earlier literature on the subject. It may be mentioned that almost every author has divided the group into species in a different way, and using different criteria. Thus the number of species recognized varies from one to twelve in the different systems. OWNBEY's paper is the latest in this series. OWNBEY has divided the group into four species, which include

several varieties: *P. pubescens*, *P. cobrense*, *P. biflorum*, and *P. commutatum*. OWNBEY, however, emphasizes that the species vary greatly and that their identification encounters difficulties. Thus the only reliable criterion for distinguishing *P. biflorum* and *P. commutatum* would be the fact that the former is diploid while the latter is tetraploid.

A cytological examination of this group has elucidated the causes of this taxonomic confusion (THERMAN, 1950). The basic haploid chromosome number is 10. In addition to diploid forms, the material of three of the species contained polyploids. Of *P. pubescens*, *P. biflorum*, and *P. commutatum* tetraploid strains with $2n = 40$ chromosomes were found. In addition a hexaploid strain of *P. commutatum* with $2n = 60$ chromosomes was encountered. Of *P. cobrense* only one clone was available which turned out a diploid.

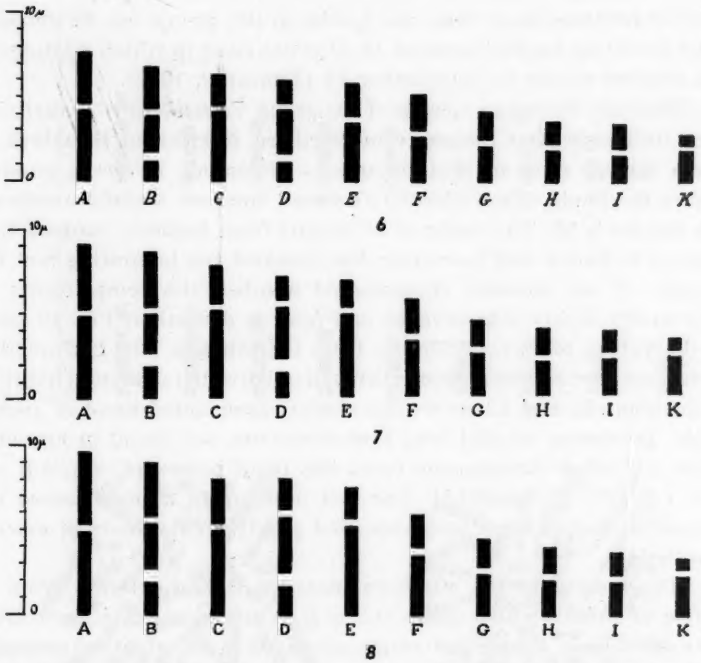
In regard to their chromosome morphology, all the species represented the *Latifolium*-type. An interesting feature in the diploid strains was that their karyotypes were similar in the various strains of one species as well as in different species. Figs. 7—8 show the idiograms of two species, *P. biflorum* from Ohio and *P. commutatum* from West Virginia. It should be noted how closely the karyotypes of these strains, from widely separated localities, resemble each other as well as *P. latifolium*.

The course of meiosis is regular both in the diploid and the polyploid forms. In the tetraploid no multivalent formation is found, the chromosomes forming only bivalents. This is the reverse of the European triploids in which most of the chromosomes form trivalents.

The similarity of the karyotypes of the American *Polygonatum* strains suggests that no structural changes, or very few of them, have occurred in their chromosomes. This conclusion is convincingly borne out by a study of their meiosis, in which practically no irregularities, resulting from structural changes in the chromosomes, could be observed.

These observations give us a key to the understanding of the taxonomical difficulties in this group. At the diploid level, differentiation obviously has resulted from gene mutations. The lack of structural changes in the chromosomes indicates that the diploid forms are not genetically isolated. It is not impossible that all the diploid strains are able to interbreed. At the diploid level no speciation has thus occurred. The diploid and tetraploid strains obviously are isolated from each other, as proved by the absence of triploids and other intermediate forms. The hexaploids constitute a third isolated group.

The present author is of the opinion that a classification of the



Figs. 6—8. Idiograms of *Polygonatum* species. — Fig. 6. *P. latifolium* (Europe). — Fig. 7. *P. biflorum* (Ohio). — Fig. 8. *P. commutatum* (West Virginia).

New World *Polygonatum* forms into species is impossible. The diploids cannot be divided into species. Neither can the species line be drawn between diploids and tetraploids, or, again, between tetraploids and hexaploids. Obviously these Solomon's Seals constitute a similar complex as, for instance, the American *Tradescantias* (ANDERSON and SAX, 1936). As in these, cytological knowledge seems to be a prerequisite for any adequate taxonomic treatment of the group.

II. GROUP VERTICILLATA.

The difference in external appearance between representatives of the groups *Alternifolia* and *Verticillata* is reflected also by their cytology. In *Verticillata* the chromosomes are smaller and the majority has subterminal centromeres. By far the most common haploid number in this group is 14. Fig. 9 shows the karyotype of *P. roseum*, which has

$2n = 28$ chromosomes. Only one species in this group, *viz. P. sibiricum* has a deviating haploid number 13. It is not clear in which relationship this number stands to the number 14 (THERMAN, 1952).

The only European species of the group *Verticillata* is *P. verticillatum*. Its distribution ranges from Northern Norway to Himalaya. In North Europe it is an Atlantic species, becoming, however, montane toward the South (FRIES, 1949). The most common somatic number in this species is 28. The study of 15 strains from habitats, ranging from Tromsö to Baden and Lausanne, has revealed the interesting fact that in spite of the constant chromosome number, the complements are structurally highly heterozygous and *inter se* dissimilar. Fig. 10 shows the karyotype of *P. verticillatum* from Copenhagen. The odd numbers of various chromosome types are an indication of the structural hybridity of the plant. In Fig. 12 we see the chromosome complement of another strain, possessing an odd long L-chromosome, not found in any other strain. Of other chromosome types this plant possessed: 3 A, 4 B, 4 C, 2 D, 4 E, 3 F, 3 G, and 4 H. The fact must again be emphasized that the chromosomes have been classified purely on the basis of external morphology.

The quality of the structural changes is best inferred from the course of meiosis which shows that at least inversions and translocations have occurred. From the combination of a constant chromosome number and the variation in structure, it can be concluded that the centromeric regions are genetically important, a reverse case from the majority of organisms.

Of *P. verticillatum* one tetraploid strain with $2n = 60$ chromosomes and two hexaploid strains with $2n =$ ca. 90 chromosomes have been encountered.

The genetical system established in *P. verticillatum* is founded on a combination of vegetative reproduction through rhizomes, and sexual reproduction through seed. Primary structural changes and secondary structural changes, due to crossing-over in the changed segments, provide a source of genetical variation in these plants. The ensuing sterility is circumvented with the aid of effective vegetative reproduction which, in turn, enables further chromosomal changes to accumulate. Gene mutations obviously have been less important than structural changes, as the strains with different karyotypes do not show a corresponding morphological differentiation. The compromise in *P. verticillatum* is thus between variation, provided by the structural changes, and stability provided by asexual reproduction (*cf.* DARLINGTON, 1939,

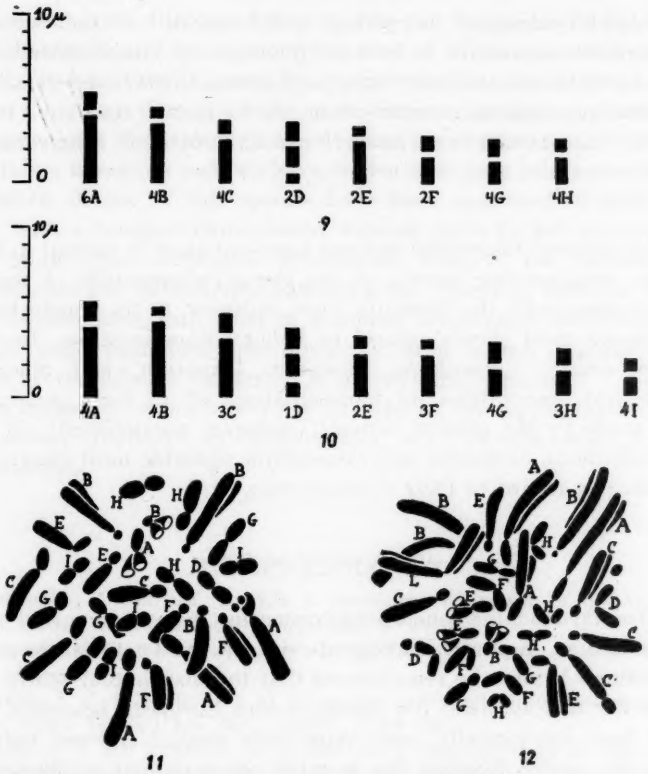


Fig. 9. Idiogram of *P. roseum*. — Fig. 10. Idiogram of *P. verticillatum* (Copenhagen). — Fig. 11. Chromosome complement of *P. verticillatum* (Copenhagen). — Fig. 12. Chromosome complement of *P. verticillatum* (Mustila).

1940). It may be mentioned that the karyotypes of *P. officinale* and *P. multiflorum* are intraspecifically very stable, differing thus from *P. verticillatum*.

III. GROUP OPPOSITIFOLIA.

The representatives of the groups *Alternifolia* and *Verticillata* differ considerably in their external morphology. Cytological evidence also gives support to the view that they are natural groups. Morphologically the group *Oppositifolia* stands, as it were, between these two. Our

restricted knowledge of its cytology is not against such an assumption, as the chromosomes are, at least morphologically, intermediate between those found in the two other groups (THERMAN, 1952). Concerning the chromosome numbers no conclusions can be drawn, since only two are known, *viz.* $2n = 30$ in *P. hookeri* and $2n = 64$ in *P. kingianum*.

In different taxonomic systems some variation is present in regard to the neighbouring genera of the genus *Polygonatum*. A thorough acquaintance with the literature leads, however, to the conclusion that the genera most closely related to *Polygonatum* must be: *Smilacina*, *Maianthemum*, *Convallaria*, *Clintonia*, *Disporum*, and *Streptopus*. Cytological observations on representatives of all these genera have been made by the present author (THERMAN, unpublished). Of these, *Maianthemum*, *Smilacina* and *Convallaria* resemble most closely *Polygonatum* in regard to their chromosomes.

IV. CONCLUSIONS.

Too far-reaching conclusions concerning the evolution in the genus *Polygonatum*, on purely cytological evidence, must naturally be avoided. In addition it must be remembered that the distribution centre of the genus lies in East Asia; the American and European branches, which have been cytologically most extensively studied, present only rays from this centre. Keeping this in mind, we may sum up the genetical processes which in the light of present knowledge have contributed to the evolution of the genus.

The Old World species of the group *Alternifolia* differ as to gene mutations. As isolation mechanisms, differences in chromosome structure are obviously effective. The basic haploid chromosome number in the group *Alternifolia* is probably 10 from which the number 9 is a secondary derivative. Polyploidy is absent except for a number of triploid hybrid forms. The chromosome complements represent either of the two types: the *Latifolium*-type, which has a much wider distribution, or the more restricted *Officinale*-type.

All the New World species belong to the group *Alternifolia* and have karyotypes of the *Latifolium*-type. Diploid American forms differ in gene mutations. Isolation mechanisms, based on changes in chromosome structure have obviously not arisen. Both tetraploid and hexaploid

forms are found. Differentiation among the American *Polygonatum* forms has obviously not reached the level at which speciation occurs, and the division of this group into species is thus impossible.

In the group *Verticillata* the most common haploid number is 14. For one species the haploid number 13 has been established. Of *P. verticillatum* a tetraploid and two hexaploid strains have been encountered. 15 diploid strains of this species have been examined cytologically. These have a constant chromosome number $2n = 28$, but their karyotypes show great structural differences. Most of the chromosome complements are structurally heterozygous. Structural changes in the chromosomes have thus been of common occurrence. Differentiation through gene mutations has obviously been of less importance, as shown by the relative similarity of the different strains.

In the group *Oppositifolia* the only known cytological facts are the chromosome numbers of two species, *viz.* $2n = 30$ and $2n = 64$ chromosomes.

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EXPERIMENTAL AND CYTOLOGICAL STUDIES ON PLANT SPECIES

I. KOHLRAUSCHIA PROLIFERA AND PLANTAGO CORONOPUS

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I. INTRODUCTION.

THIS alluring subject — the geographical distribution of cytological differences and hereditary characters within the same species — made the senior writer try to have a journey arranged during which there might be an opportunity to collect seeds or living material of a number of species from a large area of their range. This journey was realized in 1949 with a grant from the Carlsberg Foundation and the route was from Denmark to Northern Spain. The rather comprehensive material collected on the journey was later supplemented in various ways through the assistance particularly of colleagues abroad and by seed samples from wild sources obtained through botanical gardens. The large number of strains were cultivated in experimental areas attached to the Botanical Garden of the University of Copenhagen.

By communicating some of the first results of our investigations of two annual species we want to introduce a series of publications on the species in culture. The main purpose of our studies is the illustration of the genetic-biological structure of the individual species. Our wish to offer contributions of interest to the theory of evolution is of secondary importance. The discussion of the two species does not lay claim to being exhaustive. Several of the facts communicated invite further study. Supplementary experiments have already been started.

II. CHROMOSOME NUMBERS AND GEOGRAPHICAL DISTRIBUTION.

In *Kohlrauschia prolifera* (L.) KUNTH (*Tunica prolifera* SCOP.) and the closely related South European *K. velutina* (GUSS.) REICHB. mitoses in the root tips have been examined. *K. velutina* has $2n = 30$ (material from the Botanical Garden of Antwerp), while *K. prolifera* has races with $2n = 30$ and 60 in accordance with previous countings

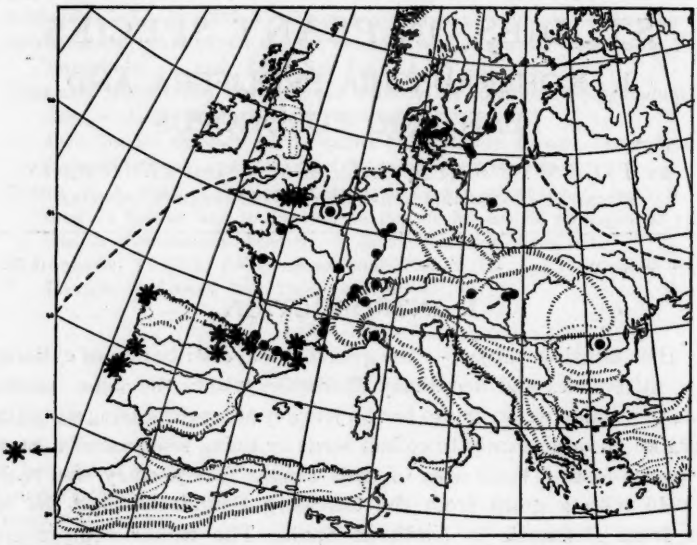

Fig. 1. Distribution of diploid and tetraploid *Kohlrauschia prolifera*. The northern limit of the species is indicated by a dotted line. Asterisks: tetraploids ($2n = 60$), dots: diploids ($2n = 30$). Dot within brackets: diploid material from a botanical garden without statement of origin. The occurrence of tetraploids in Madeira is indicated by an asterisk and an arrow pointing towards Madeira.

made by BLACKBURN (1933). The strain of *K. velutina* examined differs from the diploid *K. prolifera* by one of the chromosome pairs being much shorter than the others (see Fig. 2 *g—h* and *j—l* and FAVARGER, 1946, Fig. 12 in Plate 15). This may be a general character which may be added to the morphological differences between the two species mentioned in GUSSONE (1826), REICHENBACH (1844), and Table 1 (the size of the seeds).

BLACKBURN (1933) examined ten different stocks of *K. prolifera* four of which were tetraploid and six diploid. GENTSCHEFF (1937) and FAVARGER (1946) found diploids only. BLACKBURN's tetraploid plants originate from Spain and South England. Dr. BLACKBURN has kindly informed us the localities in question (Picos d'Europe, two stations in the Pyrenees, and Bognor in West Sussex). Furthermore, she stated having received tetraploid material from Shoreham on the Channel and Sacavem in Portugal.

The map in Fig. 1 shows the localities of all chromosome countings in *K. prolifera* made on material collected in nature and a few countings

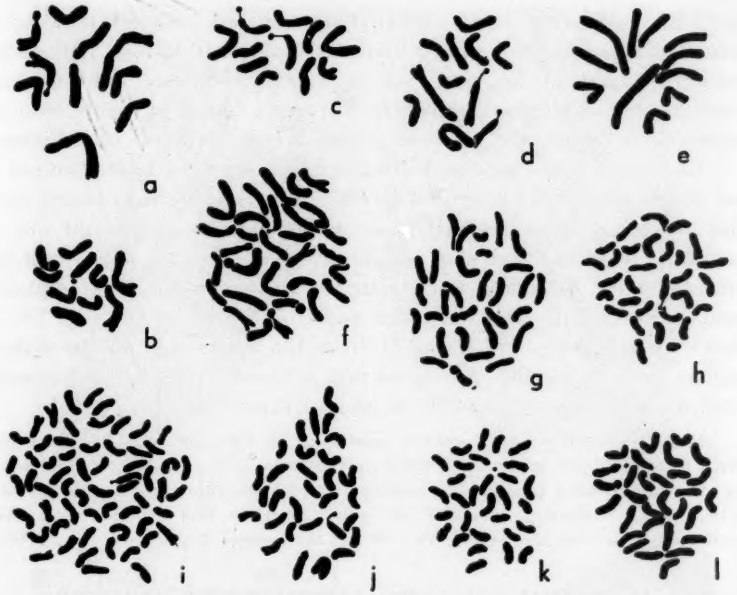

Fig. 2. Mitoses from root tips (fixation: Müntzing's modification of Navashin-Karpechenko's fixative). — *a—f: Plantago Coronopus. g—h: Kohlrauschia velutina. i—l: Kohlrauschia prolifera. a*: No. 1718 from Argeles in S. France ($2n = 10$); *b*: No. 2720 from Lincolnshire ($2n = 10$); *c—e*: No. 1351 from Mioño near Bilbao ($2n = 11$); *f*: No. 2800 from Sacavem ($2n = 30$); *g—h*: No. 913, material from the Botanical Garden of Antwerp ($2n = 30$); *i*: No. 1715 from Pont Vendres in S. France ($2n = 60$); *j*: No. 2496 from Strassbourg ($2n = 30$); *k*: No. 1696 from Mont-Louis in S. France ($2n = 30$); *l*: No. 2389 from Marburg ($2n = 30$). — × 2870.

on material from botanical gardens. 52 countings in all were made, 16 of which gave the tetraploid number. Our own material includes 40 of these countings.

The distribution of diploids and tetraploids is interesting. According to our present knowledge the tetraploids have a Lusitanian — West Mediterranean — South English distribution. The diploids occur in Central Europe, stretching towards the French side of the Pyrenees in the southwest, and alone form the advanced northern tongues of distribution reaching to the Kattegat and the southern part of the Baltic.

Plantago Coronopus L. — McCULLAGH (1934) counted $2n = 10$ in this species and the closely related *P. serraria* L. In the latter there is also an earlier counting [$n = (5)$—6] made by HEITZ (1927), and in *P. Coronopus* a counting from the Botanical Garden of Göteborg

($2n = 10$; LÖVE and LÖVE, 1944). Our material includes 16 strains from wild sources. Among these there are diploids from four stations in Denmark, three in England, six in Southern France, and one in Portugal. The deviating number $2n = 11$ was found in plants from a locality near Bilbao, and $2n = 30$ in plants from Sacavem near Lisbon.

Most species of *Plantago* belong to series with the basic number 6 and MCCULLAGH has found that *P. Coronopus* and *serraria* lack a pair with secondary constrictions. One of the pairs is large and has a median kinetochore, and one pair is satellited. Fig. 2 *f* shows root-tip mitoises of the hexaploid *Coronopus* race and Fig. 2 *a—b* of diploids from Argeles on the Mediterranean and from Lincolnshire. Fig. 2 *c—e* shows three mitoses with $2n = 11$ from the material from the Bilbao region. Particularly Fig. 2 *e* shows that it is the smallest, submedianly constricted chromosome which is present three times.

We have found a similar case of aneuploidy in *Plantago lanceolata*, of which plants from the Loire in Central France proved to have the number $2n = 13$, while the other strains have $2n = 12$. The existence of such aberrant chromosome numbers in two species of *Plantago* renders it not quite improbable that in *P. serraria* as well there may occur both the number $2n = 10$ and the number $2n = 12$ (*cf.* HEITZ, 1927).

III. MORPHOLOGY AND CHROMOSOME NUMBER.

BLACKBURN (1933) expressly states that it was not possible to point out obvious distinguishing characters between diploid and tetraploid strains of *Kohlrauschia prolifera*. Our investigations are in agreement with her statement, as we have only succeeded in finding a small difference with regard to the size of seeds, the tetraploids may have greater variation and somewhat smaller seeds than the diploids (Table 1).

In diploids as well as tetraploids the morphological variation for

TABLE 1.

Cult. No.	Species	2n	Length of seeds in mm (per cent)												n	M
			0,8	0,9	1,0	1,1	1,2	1,3	1,4	1,5	1,6	1,7	1,8	1,9		
913	*Kohlr. velutina*	30		1	31	*43*	18	7							44	1,09
1696	*Kohlr. prolifera*	30									6	16	*44*	28	6	50	1,70
1700	—	30							4	20	*50*	16	10			50	1,60
1733	—	60						2	14	26	*44*	12	2			50	1,55
1715	—	60						4	26	*30*	*30*	8	2			50	1,51
1718	*Plantago Coronopus*	10	4	*42*	*42*	12										50	0,96
2703	—	10		14	*44*	36	6									50	1,02
2720	—	10		16	24	*38*	22									50	1,06
2800	—	30	2	4	26	*54*	14									50	1,07

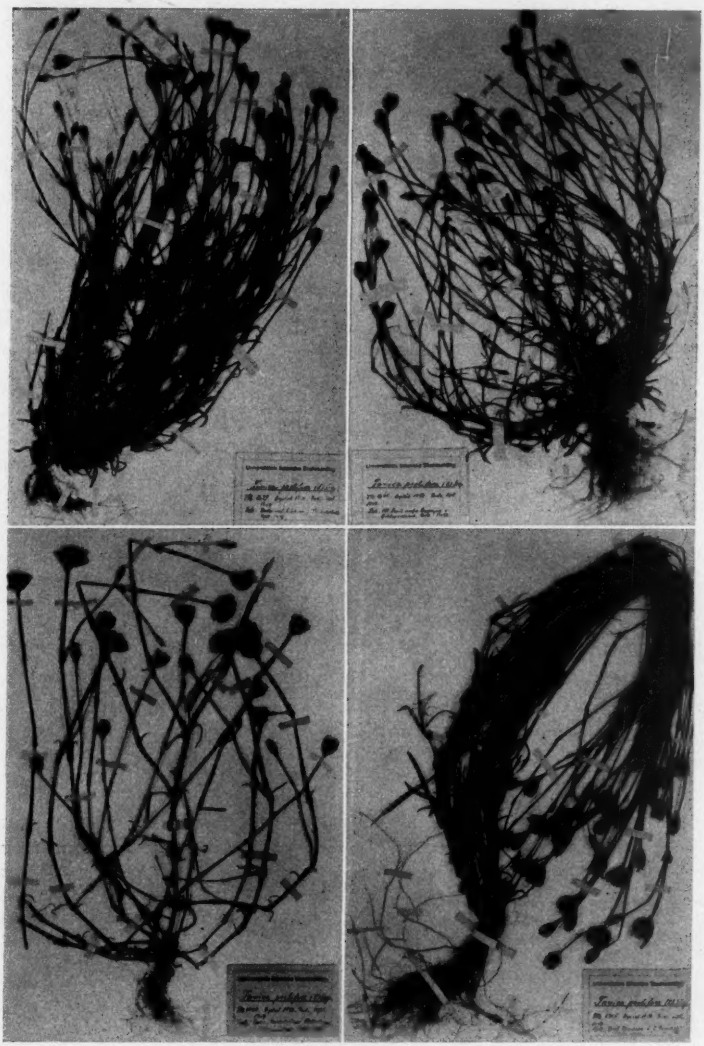

Fig. 3. Specimens of *Kohlrauschia prolifera* from a cultivation experiment in 1950. — *a*—*c*: diploids (Nos. 1627, 1696, 1700); *d*: tetraploid (No. 1715). The diploids are from different altitudes in the eastern part of the Pyrenees (see p. 299), the tetraploid is from the coastal rocks at the Mediterranean (Pont Vendres).

that matter is very great, greatest in the diploids. In both cases, however, the strains examined form character gradients. With regard to the breadth of the inflorescence (the heads) this seems to be shifted so that strains with the smallest heads are diploid and those with the broadest heads are tetraploid, No. 1700 from Southern Europe, however, being diploid and having broad heads (Fig. 3). On the other hand, our material of *K. velutina* is distinct from the diploid *K. prolifera* by a long series of characters (size of seeds, prickles on seeds, small flowers, shorter capsule, shorter cotyledons, hairiness of the stem), and so we join those authors who regard it as a particular species.

In *Plantago Coronopus* the great morphological variation has been described by PILGER (1930, 1937), who set up numerous varieties. Our cultures reveal a corresponding variation and show that it is genetically conditioned. The variation within the group of diploids, however, if anything, occurs to be continuous, as one has an impression that small leaps with regard to the characters from one strain to the other would be smaller or disappear if investigations of still more strains were available.

The hexaploid race perhaps shows a slightly greater variation in respect of size of the seeds. The size of the seeds, for that matter, is as in the dwarfish diploid strain No. 2720 (Table 1). It has rather much dwarf pollen, but besides it may be distinguished morphologically from all diploids by having larger flowers. The length of the anthers gives the best possibility of a quick distinction on a morphological basis (*cf.* Fig. 4). No. 2707 from Helgenæs in Denmark is distinct from the other diploids by having an extremely slight degree of hairiness.

An important morphological character in the diploid *P. Coronopus* is the breadth of the lobes of the leaves. The mean breadth of the middle lobe varies between 1,7 (No. 2720) and 4,9 mm (No. 1735) in the material grouped in Table 3. Two strains from the west coast of Jutland and Hammershus in Bornholm were investigated with a view to the constancy of this character. In culture the plants retained the very considerable difference, which besides breadth of the middle lobes also applied to that of the lateral lobes, the Jutland strain having long lateral lobes and the Bornholm strain had short, undivided lateral lobes. Races with very broad middle lobes and short, undivided lateral approach to *P. serraria*. Such transitional forms are frequent, *e. g.*, along the southwest coast of Spain.

IV. SIZE AND WEIGHT OF DRY MATTER IN RELATION TO CHROMOSOME NUMBER AND DISTRIBUTION.

By cultivation of a number of different strains of each of the two species under similar conditions there proved to be considerable hereditary differences in size. Material showed in the beginning of April

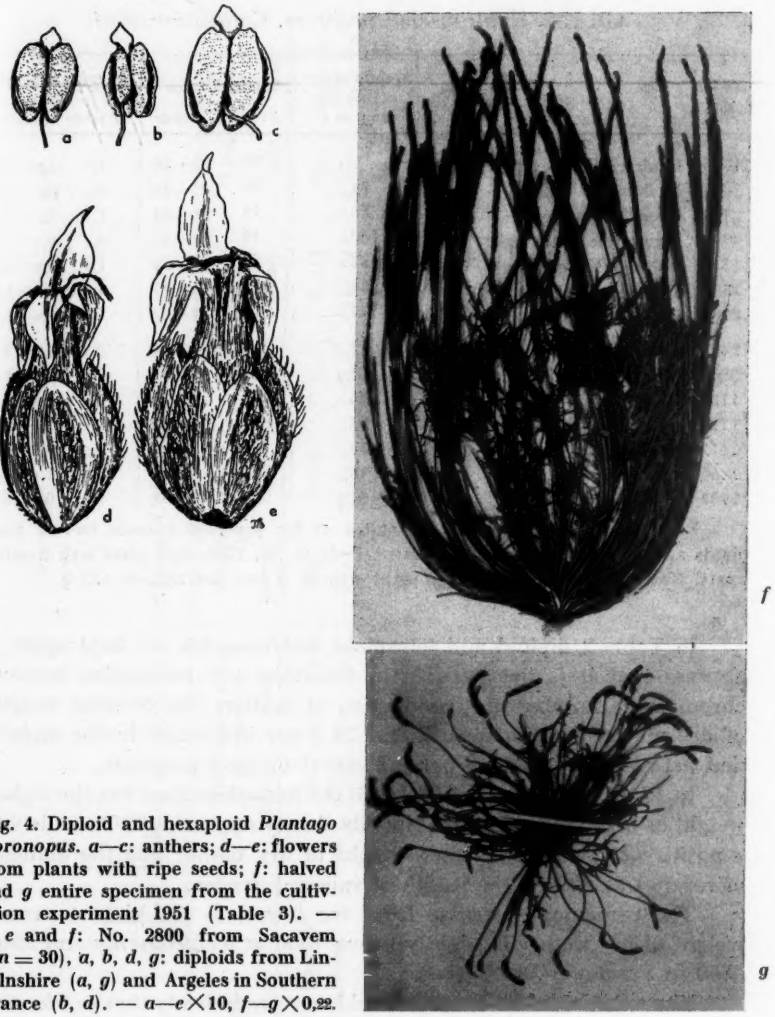

Fig. 4. Diploid and hexaploid *Plantago Coronopus*. *a—c*: anthers; *d—e*: flowers from plants with ripe seeds; *f*: halved and *g* entire specimen from the cultivation experiment 1951 (Table 3). — *c*, *e* and *f*: No. 2800 from Sacavem ($2n = 30$), *a*, *b*, *d*, *g*: diploids from Lincolnshire (*a*, *g*) and Argeles in Southern France (*b*, *d*). — *a—e* × 10, *f—g* × 0,22.

was examined in October. The sizes of the plants (in all about 20 of each strain) were measured and 5—10 plants were picked out and the parts above ground were dried and weighed. Tables 2—3 are surveys of the measurements and weighings.

TABLE 2. *Kohlrauschia prolifera. Cultivation 1951.*

Cult. No.	Origin	2n	Weight of dry matter per plant in g[1]	Height in cm		Breadth of head in cm	
				Average	Range	Average	Range
1700	Bouleternère, S. France	30	7,4	33	22—46	1,8	1,2—2,8
1627	Luchon, S. France	30	18,0	28	17—40	0,7	0,5—1,5
2762	Caen, N. France	30	25,3	44	31—53	1,7	1,0—2,5
2700	Jernhatten, Denmark	30	30,0	44	28—47	0,8	0,6—1,3
1510	Getre, S. France	30	32,4	39	14—52	1,5	0,9—2,6
2497	Graz, Austria	30	36,7	47	36—62	1,2	0,8—1,6
2785	Budapest, Hungary	30	78,5	57	42—68	1,2	0,8—1,8
2483	Coimbra, Portugal	60	13,7	45	29—62	2,3	1,4—2,7
2806	Sacavem, Portugal	60	35,2	37	21—59	1,1	0,8—1,6
1733	Beziers, S. France	60	36,9	47	29—58	1,5	0,8—2,3
1715	Pont Vendres, S. France	60	37,2	46	34—60	1,7	1,4—2,0
1472	Soria, Spain	60	39,8	49	37—64	1,2	0,8—1,8
1430	Villa Nueva, N. Spain	60	46,6	45	41—54	1,6	1,2—2,5
2763	Lisbon, Portugal	60	57,5	43	25—48	1,9	0,8—2,3

[1] Only the mean value can be stated as for practical reasons two to more plants at a time were weighed and dried. Only in No. 2785 each plant was weighed apart. Here the lowest weight per plant was 49 g and the highest 122 g.

In Table 2 diploid and tetraploid *Kohlrauschia* are kept apart. It appears that it is not possible to ascertain any connection between chromosome number and production of matter. The average weights of dry matter vary between 7 and 79 g per individual in the diploids and between 14 and 58 g per individual in the tetraploids.

In *Plantago Coronopus* (Table 3) the hexaploid race has the highest weight of dry matter, but the diploids closely approach it. There is here a positive correlation between weight of dry matter and the diameter of rosettes as well as the length of spikes + scapes.

The percentage increase from the lowest to the highest average weight of dry matter is slightly below 1000 in *Kohlrauschia* and about 3300 in *Plantago Coronopus*.

The experiment in Table 3 would seem to indicate that in *Plantago Coronopus* there is a connection between distribution and production of matter, increasingly evident towards the south. This impression is strengthened by the material in a previous culture experiment (1950), in which two strains from Denmark, five from areas on the Bay of Biscay, and four from the Mediterranean countries were examined regarding breadth of rosettes and length of scapes + spikes. The latter

TABLE 3. *Plantago Coronopus. Cultivation 1951.*

Cult. No.	Origin	2*n*	Weight of dry matter per plant in g		Breadth of rosettes in cm		Length of spikes + scapes in cm		Length of spikes in cm	
			Average	Range	Average	Range	Average	Range	Average	Range
2720	Lincolnshire, England	10	2,8	1,3— 4,9	10,0	7—14	8,6	6—13	1,7	0,8— 3,0
2640	Avernakø, Denmark	10	10,7	6,7— 12,8	20,4	16—30	18,3	11—24	5,3	3,0— 8,5
2674	Skansehage, Denmark	10	12,6	7,8— 16,3	19,1	14—24	16,9	12—23	3,8	2,0— 4,5
2707	Helgenæs, Denmark	10	27,8	18,0— 32,7	25,2	17—33	21,1	15—28	7,2	5,0—10,0
2631	Folkstone, England	10	38,3	25,1— 51,3	32,1	27—40	26,3	24—32	8,7	6,0—12,0
1312	Les Pesquite, S. France	10	39,4	13,7— 53,0	24,0	15—39	21,2	14—29	5,6	3,0— 8,0
1718	Argeles, S. France	10	82,8	57,6—107,5	35,9	(17)—46	30,3	(9)—37	9,1	3,5—13,0
1735	Beziers, S. France	10	87,6	61,6—116,2	44,1	33—50	36,8	28—44	10,6	8,0—17,0
2800	Sacavem, Portugal	30	95,6	72,6—116,4	38,8	30—48	32,3	25—39	11,2	6,0—15,0

measurement for the two Danish strains gave an average of 16 cm, for the five from the Bay of Biscay 19 cm, and for the four Mediterranean strains 30 cm. Among the five from the Bay of Biscay was No. 1351 from Mioño near Bilbao with $2n = 11$. This strain had nothing remarkable about it; its chromosome number was not known at the time when the plants were measured; so the culture was not continued the following year.

In *Kohlrauschia prolifera* there is no distinct geographical distribution of races with different production of matter. There is a tendency towards diploids from northern regions and mountains in Southern Europe (Nos. 1627, 2762, 2700, and 1510) having lower production of matter than the majority of the southern tetraploid strains. The breadth of the heads increases among diploids towards the south, and from mountainous regions in the Pyrenees (Luchon) towards Mediterranean regions farthest east in the Pyrenees. (Fig. 3.)

V. LIFE FORM AND EARLINESS IN RELATION TO CHROMOSOME NUMBER AND DISTRIBUTION.

Kohlrauschia. — An earlier experiment (1947) showed that *K. velutina* (No. 913) was very early as compared with six diploid strains of *K. prolifera*. In the later experiments in 1950 and 1951 a great difference was discovered within *K. prolifera* regarding the speed of development of the plants. The slowest plants remained in a leafy stage of reinforcement and did not succeed in flowering after sowing of the seeds in the beginning of April. The fastest had hardly any leafy stage and flowered as early as in the beginning of July (No. 1700). The strains which in the autumn had not succeeded in flowering or flowered very late with few flowers, in certain cases were capable of wintering. Still, it was always a very small number of the plants which would survive the winter. In the experiment of 1951 only 4 out of 20 vigorous vegetative individuals of No. 2801 from Strassbourg were alive after the fairly mild winter of 1951—52, and only 2 out of 20 of No. 2764 from Nantes survived. All the other strains in the experiment (Table 2) flowered after which all individuals died.

Table 4 shows life form and earliness in relation to chromosome number and geographical distribution in the experiment of 1950, which comprised 19 strains. Southern races are earliest. Only northern diploids occasionally succeed in becoming biennial. Most purely or partly vege-

TABLE 4. *Kohlrauschia prolifera. Experiment 1950.*

		Number of strains			
		Diploids $2n = 30$	Tetraploids $2n = 60$	Northern[1] (north of lat. 45° north)	Southern[1] (south of lat. 45° north)
Plants remain vegetative		1	1	1 (1)	1 (0)
Some plants quite vegetative, others with late flowering		3	0	3 (2)	0 (0)
All plants succeed in flowering	Late	5	0	3 (1)	2 (0)
	Intermediate	3	2	0 (0)	5 (0)
	Early	1	3	0 (0)	4 (0)

[1] Figures within brackets: number of strains in which a few individuals winter and become biennial.

tative strains are northern. The one southern diploid which would not flower (No. 2539 from Lisbon) perhaps is a short day race.

The experiment of 1950 included three strains from different altitudes in the Pyrenees (Fig. 3). These were all diploids and flowered abundantly. No. 1627 from Luchon (mountainous) was a multicauline and leafy type which began flowering on August 12 (Fig. 3 *a*). No. 1696 from Mt. Louis (nearer to the lowlands) was less leafy, with many ascending flowering stems and began flowering on August 7 (Fig. 3 *b*). Finally, No. 1700 from a Mediterranean maquis at Bouleternère was a deviating race with a very open structure of shoots and few stems and leaves, which flowered on July 5 (Fig. 3 *c*).

Plantago Coronopus. — In the experiment of 1950 with 8 southern strains and 2 northern (Danish) ones, all diploids, 5 were early and 5 somewhat later, among them the two northern strains.

The experiment of 1951 comprised 9 strains (Table 3). There was little difference between them in respect of earliness, but there were interesting differences as regards wintering, Nos. 2720, 2707, 2674, and 2640 from Denmark and England (all northern strains) had a number of wintering individuals, while there was only one such in No. 1312 from Southwest France and none in the rest of the southern strains and No. 2631 from England. In the last-mentioned strain the first rosette-leaves were ascending as in most plants of southern origin, while otherwise in all northern strains the rosette-leaves were adpressed

Fig. 5. *Plantago Coronopus*, No. 2720 from Lincolnshire. The plant appearing after being sown in April, 1951, and dug out for being drawn on April 19, 1952. The vertical short rhizome has branched at the end of the first vegetative period and has formed four rosettes, two of which are seen in the drawing. The lowest (oldest) rosette-leaves have been exposed to the winter cold and have withered at the tips.

to the ground. Two other strains from Denmark, cultivated in pots, had, respectively, 66 and 87 per cent wintering plants. As in *Kohlrauschia prolifera*, there is thus a clear increase in a northerly direction in the faculty of wintering. In *Plantago Coronopus* it is probably often a question of perenniality, as several of the surviving plants have branched and formed several rosettes, as constantly in the case of the Mediterranean *Plantago macrorhiza* POIR, which is closely related to *P. Coronopus*. A wintering individual of the dwarfish race from Lincolnshire (No. 2720; see Table 3) is pictured in Fig. 5.

VI. DISCUSSION AND CONCLUSIONS.

(1) In both species discussed there is polyploidy and in both the highest chromosome number is found in the south. The instances join the not quite small number of other species (*e. g.*, *Vaccinium uliginosum*, HAGERUP, 1933; *Campanula rotundifolia*, BÖCHER, 1936) which go against the theory of an increase in the degree of polyploidy in a northerly direction (the HAGERUP—TISCHLER theory, which has recently been discussed in detail by LÖVE and LÖVE, 1949). It should also be pointed out that in both species the diploids have the largest area of distribution, which does not correspond to the majority of known distributions of diploids and tetraploids. Furthermore, it is the diploids which have been capable of invading previously glaciated areas (cp. MANTON's remarks, 1950, pp. 282—83).

(2) Both species examined are annual or exceptionally bi- or perennial. All southern strains are strictly annual, thus all tetraploids of *Kohlrauschia* and the hexaploid *Plantago Coronopus*. An increase in

the degree of duration of life in a northerly direction has been described in *Prunella vulgaris* by BÖCHER (1949) and corresponds to RAUNKIÆR's ascertainment of the low percentage of annuals in Northern Europe; cp. RAUNKIÆR, 1934, p. 122. The fact that the polyploid strains are strictly annual, while some of the diploids show a tendency towards bi- and perenniality is a slight corrective of MÜNTZING's ascertainment (1936) of a positive correlation between annual life and low chromosome number. Annuals with a chromosome number above 40 were not found in any of the cases examined by MÜNTZING, and only two annuals exceeded the number 30. Later, WULFF (1937) has counted $2n =$ about 120 in the hapaxanthous species *Juncus ranarius*. So far this species and *Kohlrauschia* are the only hapaxanthous species exceeding the chromosome number 40. But even if the hexaploid *Plantago Coronopus* with $2n = 30$ is added, these three examples are far from sufficient to shake the correspondence found by MÜNTZING (cp. also remarks in GUSTAFSSON, 1947).

(3) Whereas it may be possible on the basis of morphological characters to set up the hexaploid *Plantago Coronopus* as an independent species (or subspecies), it is not possible to divide *Kohlrauschia prolifera* into a diploid and a tetraploid species. LÖVE, 1951, p. 283 writes: »Morphologically completely indistinguishable polyplotypes are unknown, at least in northwestern Europe». If England is included in Northwestern Europe, *Kohlrauschia prolifera* was already by BLACKBURN (1933) mentioned as an instance of a Northwest European species with indistinguishable polyplotypes. Our investigations confirm BLACKBURN's result as we are not of opinion that so small a difference as the one mentioned regarding the size of seeds can form the basis of a distinction between species. Even if the difference found should prove constant at future measurements of seeds, it would appear unnatural to draw a boundary-line on the basis of a difference of this order of magnitude, particularly as the genetic morphological variation in diploids as well as tetraploids is very considerable, including so greatly deviating types (*e. g.*, No. 1700, Fig. 3) that one might feel inclined to consider them as independent species. So we cannot accept the view of LÖVE (1951) that »all types differing in the number of chromosomes should be classified as distinct species». We admit willingly that *Kohlrauschia prolifera* comprises two genetic separated units. They correspond to what DARLINGTON (1939) and WESTERGAARD (1943, p. 53) mean by the term crypto-species and to what CLAUSEN (1951, Fig. 70) calls genetic species. Altogether a division into taxonomic species on the

basis of the chromosome number is not nearly so urgently required as a distinction considering greater qualitative genetic differences. If the two chromosome sets of an autopolyploid plant has not during the time of its existence undergone any material changes and thus on the whole are alike, the plant will probably be more closely related to its diploid ancestor than the latter will be to another diploid which is distinct by a number of qualitative properties, this in spite of the fact that in the first case there probably will be a sterility barrier, while the two diploids perhaps are interfertile, but geographically and morphologically distinct. There is greater reason to distinguish the diploid *Kohlrauschia velutina* from the diploid *K. prolifera* than to distinguish the polyplotypes within the latter. A difference in the size of chromosomes which in this case has been found between the two diploid species may be connected with the existing qualitative differences and thus presumably a greater importance must be ascribed to it than to a doubling of a chromosome set.

(4) The very considerable genetic differences regarding the production of dry matter in a number of strains with the same chromosome number of two annual species (Tables 2—3) are of a certain interest as compared with annual cultivated plants where something corresponding is hardly known. This contrast, however, is presumably due to the fact that man has long ago selected the races of cultivated plants which have the highest production.

Hereditary differences in the production of dry matter of the same order of magnitude as that described in *Kohlrauschia* and *Plantago Coronopus* have been found among perennial herbs (*e. g. Veronica officinalis*; see Table 1 in BÖCHER, 1944). The great differences in growth or height found in different races or proveniences of trees also suggest that here there are considerable differences in the production of matter, although they are hardly quite so great as, *e. g.*, those in *Plantago Coronopus.*

It was not possible to establish any general difference as regards the production of dry matter between tetraploid and diploid *Kohlrauschia* and the difference found in that respect between hexaploid and diploid *Plantago Coronopus* is insignificant as compared with the differences between the diploids.

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CHROMOSOME FRAGMENTATION AND ACCESSORY CHROMOSOMES IN ORTHOTRICHUM TENELLUM

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IN three earlier papers the present author has described three moss species in which a normal chromosome set was complemented with a few tiny chromatic bodies which were considered true accessory chromosomes on the basis of their characteristics and meiotic behaviour. In addition to these three species, *viz. Grimmia Mühlenbeckii* SCHIMP. (VAARAMA, 1949), *Ulota curvifolia* (VG) BRID. (VAARAMA, 1950 a) and *Dicranum majus* TURN. (VAARAMA, 1950 b), a similar type of chromosome set has been observed later in a peat-moss, *Sphagnum squarrosum* PERS. (VAARAMA, unpubl.).

WHITE (1951, p. 357), in his definition of accessory chromosomes, says that they are »elements which are present in some individuals of the population but not in others». Because individuals devoid of accessories have thus far not been found in the investigated populations of the moss species mentioned above, the minute chromosomes observed cannot be included in the category of true accessory chromosomes, as defined by WHITE. The great similarity between the moss-accessories and some types of accessory chromosomes known in the animal (*cf.* MELANDER, 1950; WHITE, 1945, 1951) as well as in the plant kingdom (RESENDE and DA FRANCA, 1946; ÖSTERGREN, 1947) leads, however, to the assumption of a close kinship, at least as to their origin. Thus it seems that the moss-accessories cannot be excluded from the category of true accessories even in the strict sense of terminology. Moreover, because only a very fragmentary part of populations belonging to the moss species referred to has been investigated thus far, it is reasonable to expect that individuals without accessories may also be found in the future.

During the winter of 1952 accessories of the same tiny chromosome type were found again in a new moss species, *Orthotrichum tenellum* BRUCH. The moss was found by the author growing on the trunk of a Coast Live Oak, *Quercus agrifolia*, in that part of Stanford University Campus, Stanford, California, which is called the Cactus Garden. Because the original purpose was to make a chromosome number

determination, preparations were made only from a few sporogones. Later on, when it appeared desirable to perform more comprehensive investigations, the collection of more material was no longer possible.

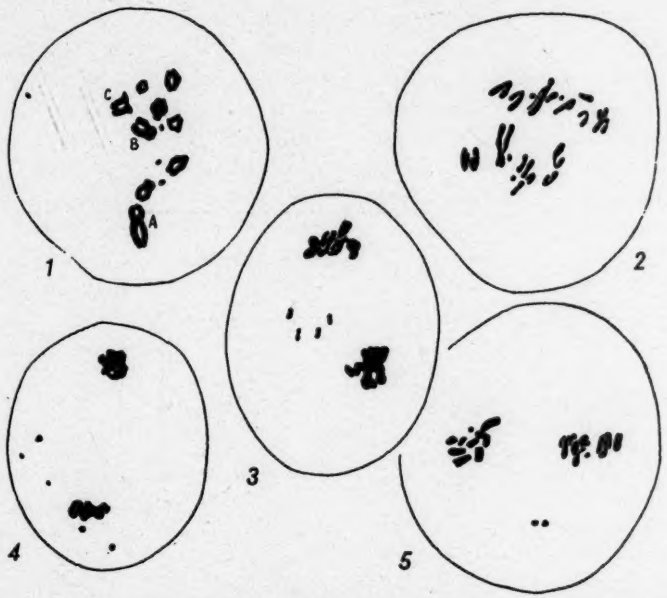
Because some of the sporogones prepared contained a great number of spore mother cells at various stages of meiotic division, it was possible to obtain a fairly satisfactory although not complete picture of the structure and behaviour of the minute accessories as well as of the ordinary chromosomes.

Squash preparations were made using propionic orcein as fixing and staining agent. The preparations were later made permanent. The original drawings were made using Abbe's camera lucida, 114× oil immersion, apert. 1,32, and 30×eyepiece. The magnification was 5500×.

OBSERVATIONS.

Ordinary bivalents. — Among the sporogones prepared there were only two in which the stages of meiosis were suitable for cytological analysis. One of them (called henceforth sporogone 1) had nine bivalents (Fig. 1), whereas in the other (called sporogone 2) their number was ten (Fig. 6). This difference in bivalent number was constant in the numerous spore mother cells (SMC) which were at a suitable stage for counting and could be determined without any possibility of error.

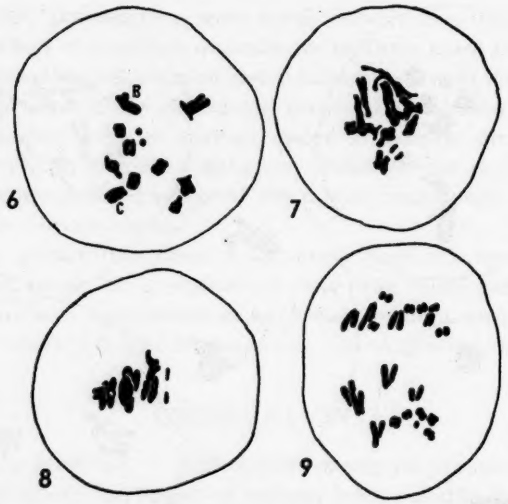
The morphological comparison of the bivalents in the two sporogones revealed that the complement of sporogone 1 is characterized by the presence of one large bivalent (A-bivalent) having submedian constriction (Fig. 1). This bivalent type was lacking in sporogone 2. The same difference between the chromosome complements is seen in Figs. 2 and 9 which present first anaphases. The two halves of the long A-bivalent are rather dominating in Fig. 2. Chromosomes of the same size class are absent in Fig. 9. In Fig. 6, position about 2 o'clock, a bivalent is visible the size of which corresponds well with the length of the longer arm of the A-bivalent. On the other hand, there are two additional bivalents in the sporogone 2 which could be identified with corresponding ones in the sporogone 1 (Figs. 1 and 6, bivalents B and C). The rest of bivalents consisting of rather small types of about equal size cannot be kept separated but their number is seven in sporogone 2, the corresponding number in the sporogone 1 being six. There is reason to suppose that the small extra bivalent in sporogone 2 corresponds to the short arm of the A-bivalent. The length of the small bivalents is very close to that of the short arm.



Figs. 1—5. *Orthotrichum tenellum*, sporogone 1. — Fig. 1. First metaphase plate with 9 ordinary bivalents and four minute accessories, one of them outside the spindle. — Fig. 2. First anaphase. Even distribution of accessories. Halves of A-bivalent very conspicuous. — Fig. 3. Late first anaphase. Four lagging accessories. — Fig. 4. First telophase. Five eliminated bodies. One of the lagging accessories divided. — Fig. 5. Second metaphase. Two eliminated accessories. — Propionic orcein squashes. Magnif. 3700×.

On the basis of above observations it seems most probable that the increased bivalent number in sporogone 2 is due to fragmentation of the long A-bivalent present in sporogone 1. The fragmentation has obviously taken place through the submedian constriction present in the A-bivalent, and the two new bivalents formed correspond to the arms of the long chromosome. It may also be stated as likely that the A-bivalent is a fusion product of the two bivalents mentioned present in sporogone 2.

Accessory chromosomes. — In the two sporogones (1 and 2) investigated there were visible a few minute, but distinctly staining, chromosomes quite similar to those found earlier in the moss species *Grimmia Mühlenbeckii* and *Ulota curvifolia*. Their number was constantly four in sporogone 1 (Fig. 1) but in sporogone 2, in all cases, two (Fig. 6).



Figs. 6—9. *Orthotrichum tenellum*, sporogone 2. — Fig. 6. First metaphase plate with 10 ordinary bivalents and two minute accessories. — Fig. 7. Diakinesis. The two accessories, 5 o'clock position, show distance conjugation. — Fig. 8. First metaphase side view. Distance conjugation of the accessories. — Fig. 9. First anaphase. Regular distribution of accessories. — Propionic orcein squashes. Magnif. 3700×.

In the large diploid nuclei of columella two large bodies were present that show heteropycnosis and, in addition, smaller dot-like bodies the number of which was in suitable cases found to be four in sporogone 1 and two in sporogone 2. It is likely that the small heteropycnotic bodies correspond to the accessory chromosomes observed in meiotic divisions. It is evident that these are heterochromatic as to their main characteristic.

The heteropycnotic nature of the accessories disappears at meiotic prophases to such an extent that they are no longer discernible during these stages of division. The same is true also regarding the large heterochromatic chromosome pair represented in somatic nuclei by a pair large heteropycnotic bodies. The accessories of *O. tenellum* thus correspond in their type of allocycly completely with those observed in the moss species *Grimmia Mühlenbeckii* (VAARAMA, 1949) as well as in the minute accessories of the grass *Anthoxanthum* (ÖSTERGREN, 1947).

The chromatinization of the accessory chromosomes advances in pace with that of other chromosomes. During the stage which best may

be called diakinesis (meiotic chromosomes lie clumped together more or less compact in the middle of nucleus, a type which seems to prevail in mosses) the accessories are already clearly discernible (Fig. 7).

It is henceforth most suitable to follow the behaviour of the accessory chromosomes separately in the two sporogones in question.

The congression of the four minute accessories to the equatorial plate at the first metaphase is in many cases irregular in the sporogone 1 (Fig. 1). One or more of them lie outside the plate. The anaphase separation of the halves of ordinary bivalents is regular. There was among the 100 SMC's investigated only four cells showing one lagging bivalent. The frequency of lagging minute accessories is seen in Table 1.

TABLE 1. *Frequency of lagging accessory chromosomes in sporogone 1.*

Stage	% normal divisions	% varying number of lagging accessories					Total number of cells
		1	2	3	4	5	
1st ana-, telo-phase	59	20	16	2	1	2	100
2nd ana-, telo-phase	66	21	13	9	—	—	103

The distribution of the accessory chromosomes at the poles may be quite regular, two and two, as is seen in Fig. 2. It is obvious that the accessories do not undergo division during the first anaphase. This is, however, a rule in the second division. The lagging of the accessories may, however, change the situation so that there sometimes occurs delayed division of an accessory at first anaphase (Fig. 3). It is accordingly possible to see more than four lagging accessories between the telophasic groups (Fig. 4). The maximum number of laggards observed is, however, only five and, as seen in Table 1, also the frequency of such cases is very low.

Irregularities in the behaviour of accessories continue in the second division. Sometimes a few of them remain outside the spindle at the second metaphase (Fig. 5). The most common type of irregularity is, however, the lagging of a few accessories between the anaphasic chromosome groups. The frequency of various numbers of laggards is presented in Table 1.

On the basis of the observations made it is obvious that approximately 35 per cent of SMC's in the sporogone 1 have suffered the loss of one, two, three or all accessories. It is clear that also the distribution

of accessories in the SMC's included in the category of normals in Table 1 is not always two and two. Distributions 3 + 1 and 4 + 0 are in fact sometimes observed.

In spite of the irregularities observed the spores formed in tetrads are morphologically quite perfect. The few abnormalities met with are evidently due to some exceptional disturbances in the division of ordinary bivalents.

As has been mentioned, only two minute accessories are seen in sporogone 2. In some fortunate cases it has been possible to distinguish them also at the stage of clumped diakinesis (Fig. 7). At this stage accessories are always located close to each other like mitotic chromosomes showing somatic pairing. There are no visible connections between them. The congression of the pair to the metaphase plate is quite normal. Accessories of sporogone 2 have never been observed outside the equatorial plate. The proximity of the minute accessories is preserved at metaphase. They are located opposite each other on both sides of the equatorial plate but are not in contact (Fig. 8). The approximate distance between the two bodies in metaphase side views is about 3—5 times the diameter of one accessory. This type of meiotic conjugation may be called distance conjugation according to the term first proposed by LORBEER (1934) who observed the phenomenon in several liverwort species. A similar condition regarding one chromosome pair has also been described in the moss *Hedwigia ciliata* (VAARAMA, 1950 a).

The behaviour of the pair accessories at first anaphase is as regular as that of the ordinary bivalents. The percentage of SMC's with lagging first division accessories is only 1,7, *i. e.*, exactly the same as that of the SMC's with lagging ordinary bivalents. The maximum number of lagging minute accessories is one.

The separation of the accessories at first anaphase is, as a rule, according to the regular 1 : 1 ratio (Fig. 9). In some few cases, however, also the non-disjunctional 2 : 0 ratio may be realized.

The regular behaviour of the two accessories is again visible at the anaphase of second division. The percentage of SMC's showing one lagging accessory is two at the same time as the percentage of SMC's with lagging ordinary chromosomes is three.

The tetrads formed in sporogone 2 are according to morphological investigation quite regular and uniform.

The frequency of lagging accessories has also been estimated from a third sporogone full of SMC's uniformly at the second telophase

stage. 250 SMC's were examined and among them there were only two exhibiting three lagging minute chromosomes scattered between the telophasic nuclei. The percentage of cells with laggards is accordingly 0,8.

DISCUSSION.

Chromosome fragmentation. — The observation that the number of normal bivalents may vary in different individuals of the same population of *Orthotrichum tenellum* is a very interesting one. It may be stated on the basis of morphological observations made that we have a rather convincing case of chromosome fragmentation-fusion. The big A-bivalent of sporogone 1 has broken in two and the parts, which have not lost their mobility, are seen in sporogone 2.

This characteristic makes it very improbable that the increase in chromosome number has taken place by means of some usual type of structural change in the chromosomes. There is, on the other hand, no evidence in favour of an assumption that the centromeres in this moss species were of the diffuse type found, *e. g.*, in *Luzula* (MALHEIROS, DE CASTRO and CAMARA, 1947) or in coccids (HUGHES-SCHRADER, 1948).

It may be seen directly that the longer part of the fragmented A-bivalent is telocentric and the same is most probably true also of the shorter part. The chromosome breakage has thus most likely taken place through the primary constriction visible in the A-bivalent of sporogone 1. The case is analogous with the usual misdivision of a centromere (*cf.* DARLINGTON, 1939), differing, however, in the essential respect that both fragments have a perfect centromere and maintain their capacity to undergo a completely normal division.

This type of fragmentation may be explained by assuming that the A-bivalent of the sporogone 1 is a fusion product of two telocentric (or perhaps more exactly, almost telocentric) rods with their kinetic ends together. There are thus two fully perfect centromeres quite close to each other, separated only by a narrow fusion zone which probably constitutes the main part of the primary constriction in the A-bivalent. If the fragmentation occurs at the fusion zone — and we have reason to believe that in usual conditions it occurs just at this point — there would arise two chromosomes with perfectly functioning centromeres.

Cases comparable with that described may be found in grasshoppers which insect group is characterized by a frequent fusion of two rod chromosomes to a V-shaped one, a phenomenon which has led to the establishment of the well-known law of ROBERTSON (*cf.* WHITE, 1945).

The existence of two perfect, adjacent centromeres at the primary constriction of the V-shaped orthopteran chromosomes has been suggested, *e. g.*, by COREY (1933) and HELWIG (1933). This assumption has received further support from the more recent observations of ROTHFELS (1950).

The existence of the double centromere mechanism suggested above for the A-bivalent of *O. tenellum* is most likely also on the basis of other observations made by the present author in several other moss species, particularly in *Pleurozium Schreberi*. A more detailed discussion of these observations has, however, been omitted in this connection because a rather comprehensive paper is just under preparation.

Accessory chromosomes. — The two minute chromosomes found in the sporogone 2 are quite comparable with the ordinary bivalents in their meiotic behaviour. Their congression to the first metaphase equatorial plate and their anaphase segregation are very regular. They differ, however, from the ordinary bivalents in their size, somatic heteropycnosis and a peculiar type of pairing.

It seems possible to identify this minute chromosome pair with the small heterochromatic »m»-bivalent having a fairly wide distribution among the bryophytes. This chromosome type was originally detected and named by HEITZ (1927). He saw them in some liverwort species, as did also LORBEER (1934). In a later paper HEITZ (1928) postulated that the »m»-chromosomes of liverworts are always connected with sex determination. Sex-chromosomes of the »m»-type have been met with also in a few moss species (*cf.* JACHIMSKY, 1935).

According to LORBEER (1934), the »m»-chromosomes of liverworts have in several cases shown distance conjugation at the first meiotic metaphase. It is interesting to note that distance conjugation connected with sex-chromosome system has been encountered also in some insect groups. The phenomenon has, however, been called »directed disjunction» by WHITE (1940). It is known in some neuropteran and heteropteran species (*cf.* WHITE, *l. c.*) and in a couple of gryllid species from the group of Orthoptera (RAYCHAUDHURI and MANNA, 1951).

The moss species *O. tenellum* is autoik (*cf.*, *e. g.*, JENSEN, 1939), *i. e.*, male and female sex organs are located on the same individual. We do not know, therefore, whether the »m»-bivalent in *O. tenellum* has something to do with sex determination. It is all the more difficult because there is, in addition, a heterochromatic bivalent among ordinary ones. It is heteropycnotic only at the somatic resting stages and thus indiscernible in meiosis. HEITZ (1928) and JACHIMSKY (1935) have

detected heterochromosomes in unisexual bryophytes and the first-mentioned author has made a statement that these chromosomes have a sex determining function. Regarding *O. tenellum*, we have no data warranting immediate agreement with HEITZ's opinion. There might exist, however, a close relationship, between »m»-bivalent and sex-chromosomes also in this case.

We have among the mosses a case which is fully analogous with that in *O. tenellum*. An autoik species, *Hedwigia ciliata*, has a small heterochromatic chromosome pair showing distance conjugation. In addition there is a heterochromatic ordinary bivalent that cannot be identified in meiosis owing to its type of allocycly (VAARAMA, 1950 a). The small chromosome pair reveals in its development (VAARAMA, unpubl.) a close relationship with a peculiar heterochromatic bivalent observed in several pleurocarpic moss species in meiosis and called preliminarily »special bivalent» (VAARAMA, 1950 a). It develops at meiotic prophase stages on the surface of a nucleolus-like body which disappears later on. Although the decisive evidence is still lacking, it seems obvious that at least in some dioecious pleurocarpic mosses the »special bivalent» includes the sex determining mechanism and is thus comparable with »m»-type sex-chromosomes in other bryophytes.

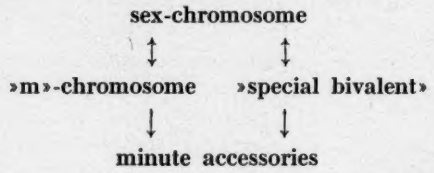
There is no doubt that the four minute chromosomes observed in sporogone 1 of *O. tenellum* are derived from the »m»-chromosome pair of sporogone 2. Evidently fragmentation into two pieces has taken place in both members of the »m»-pair. Fragmentation seems to have prevented completely the distance conjugation observed in the whole chromosomes. At the same time the kinetic power of the centromeres appears to be weakened considerably which is shown by the irregularities in their meiotic behaviour.

Owing to the minute size of the accessories in *O. tenellum* it is impossible to see the manner in which the fragmentation has taken place. However, each of the pieces formed has a kinetic element of its own. Because of the weakening of the centromere this case is not fully comparable with the fragmentation of the ordinary chromosomes as seen in the A-bivalent of sporogone 1. Perhaps a true misdivision of the centromere is involved.

The four fragments in question are in all their features identical with true accessory chromosomes and the only thing which prevents us from including them directly in this category is that we do not yet know whether or not they are found in all members of the population. On the other hand, it may be mentioned that MÜNTZING (1948) has not

hesitated to give the name accessory to the extra chromosomes found in *Poa alpina* although these were present in all members of the population studied.

It seems possible that we have here at hand a case able to reveal the type of origin of at least one, perhaps rather common, type of accessories in mosses. The accessories arise through fragmentation from heterochromatic »m»-chromosomes. Because there exists a close kinship or, in some cases, full identity between »m»-chromosomes, sex-chromosomes and the »special bivalent» we may construct a diagram to indicate a possible origin of moss accessories.



It is improbable that accessories can arise directly through fragmentation of small sex-chromosomes because of the serious consequences of this event. Moreover, if the function of sex-chromosome has been transferred for some reason or other to another heterochromatic chromosome pair present in the complement, the »m»-chromosomes or »special bivalents» may more easily function as a source of accessories through fragmentation. The functionless, heterochromatic sex-chromo somes might be expected to be fairly inert as to their genetic activity. This assumption is not, however, proved because moss individuals without accessories have not thus far been found.

This type of origin of accessory moss chromosomes resembles in many respects that of certain animal accessories. Their close relationship with the sex-chromosome system has been observed several times (*cf.* MELANDER, 1950; WHITE, 1951). In general, it may be stated that concerning the minute accessories in mosses the closest analogies are to be found in animal accessory chromosomes rather than in those of higher plants. It is obvious that there are also other types of origin of moss accessories. The development of the five accessories present in the meiotic cells of *Dicranum majus* and characterized by frequent ring and chain formation (VAARAMA, 1950 b) cannot be directly explained on the basis of the above.

There is obviously some variation in the behaviour of the minute accessories in mosses. In *Grimmia Mühlenbeckii* (VAARAMA, 1949) the

four small accessories present have in some degree maintained their ability to pair in meiosis. Thus, the possibility is not excluded that the four accessories represent only univalents of two »m»-chromosomes with normal pairing behaviour. On the basis of the frequent lack of pairing it may be suggested that we have here an initial phase of accessory development. This development would be completed by the total, or almost total, disappearance of pairing.

I am greatly indebted to Dr. WILLIAM C. STEERE, Stanford University, Stanford, California, for the identification of the moss species studied, and to Mr. SAM PRICE, University of California, Berkeley, California, for his generous assistance in making the permanent preparations.

SUMMARY.

(1) Meiosis in spore mother cells of two sporogones of the moss *Orthotrichum tenellum* BRUCH was studied.

(2) The sporogone 1 was characterized by the presence of nine bivalents — one of them was large (A-bivalent) — and four chromosomes of minute size. The minute chromosomes are heterochromatic and show a number of irregularities in meiosis.

(3) In the sporogone 2 ten bivalents — no A-bivalent was noted — and two minute chromosomes were observed. The small chromosomes were also heterochromatic but behaved regularly in meiosis and showed distance pairing at diakinesis and first metaphase.

(4) In their main features the minute chromosomes are identical with accessory chromosomes known from other organisms.

(5) The numerical variation in the ordinary bivalents is suggested to be a result of direct chromosome fragmentation at the probably double-structured centromere of the A-bivalent.

(6) The two accessories in the sporogone 2 are closely related with the »m»-chromosomes known from several bryophytes.

(7) The four accessories in sporogone 1 have evidently arisen through fragmentation of the two minute accessories of sporogone 2.

(8) It is suggested that an initial phase of accessory chromosome formation is here involved. The possible development of moss accessories from sex-chromosomes and other types of heterochromatic chromosomes is discussed.

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SOME EXPERIENCES FROM MUTATION EXPERIMENTS IN CHLAMYDOMONAS

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AS a complement to the studies on the biological and especially the genetical effects of radiations carried out by a Swedish research group (GUSTAFSSON, EHRENBERG, MAC KEY *et al.*) the present author started a few years ago mutation experiments in the unicellular green alga *Chlamydomonas.*

A considerable part of this first period has been spent on the elaboration of suitable experimental methods and on preparative studies in order to test the utility of the material for the various lines of investigation. This paper will, therefore, be a preliminary review of the works rather than a presentation of definite results.

Material. — The material has consisted of three sexual and heterothallic, but morphologically isogamous, species, *viz. Chlamydomonas eugametos*, the classical species of MOEWUS, *Chl. Reinhardi*, obtained from G. M. SMITH, Stanford, U. S. A., and *Chl. Moewusi*, the stocks of PROVASOLI. *Chl. eugametos* and *Moewusi* seem to belong to the same species. They copulate readily with each other and show no conspicuous morphological or physiological differences.

These algae have a very simple cycle of reproduction, well known from the works of MOEWUS and from the botanical text-books. The haploid, vegetative cells with a length of 8—12 μ reproduce themselves vegetatively through the formation of unicellular zoosporangia with a rate that increases the number of cells about 3—4 times per day. These vegetative cells are also potentially equal to gametes, and if cells from both sexes are brought together under certain conditions they copulate, fuse two-by-two, and form the diploid zygotes. These zygotes, which represent the sole diploid and immotile stage, may be brought to germination with the formation of four cells, two of each sex.

Action of x-rays and ultra-violet light. — These organisms constitute a very suitable material for studying the biological and genetical effects of various radiations. Among other things, the lethalizing effects of x-rays and ultra-violet light have been compared. A few earlier observations show that when bacteria are killed by x-rays they do not die immediately but only after a few cell divisions. This has led to

the assumption that the killing of micro-organisms with radiations implies an action on the genetical material, *e. g.*, an induction of lethal mutations or chromosomal changes which would become acute when the cells started dividing.

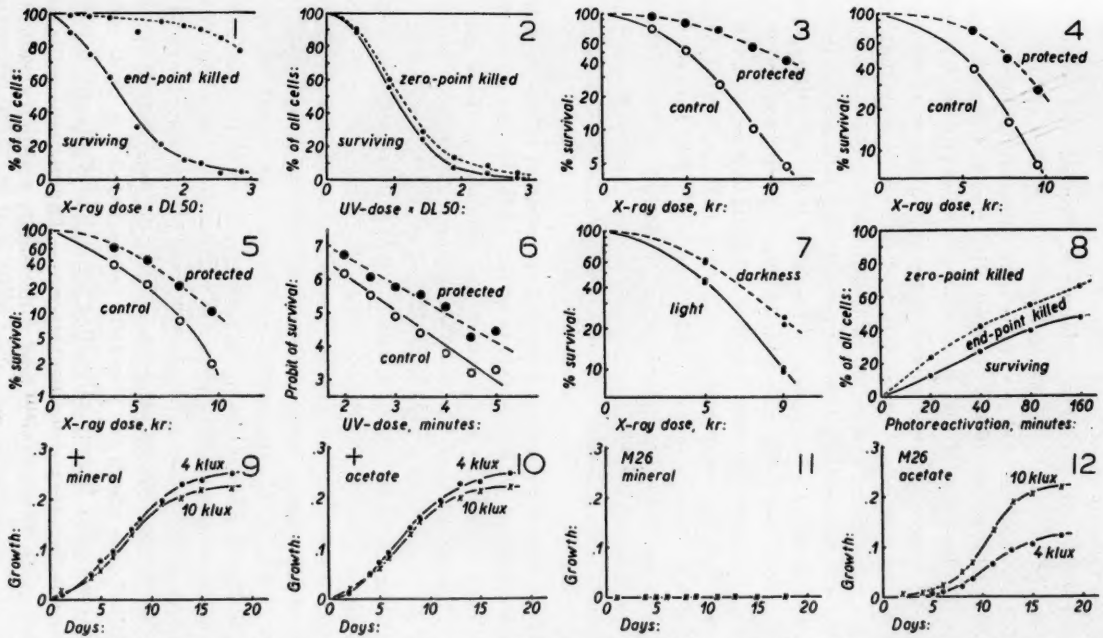
Figs. 1 and 2 show the survival curves for *Chlamydomonas eugametos* after irradiation with x-rays and ultra-violet light. The doses are expressed as functions of the semi-lethal dose. The killed cells are divided into two categories, represented by the surfaces between and above the curves, respectively; *viz.*: such cells which divide one or a few times before dying, and secondly, such cells which die immediately, without dividing. In analogy with the two types of mutations obtained in bacteria after irradiation they have been called end-point and zero-point killed cells, respectively.

Taking the x-rays first, we see that most killed cells have divided before dying, *i. e.* are end-point killed, whereas the reverse is true for ultra-violet light. Here, the end-point killed cells constitute only a minor fraction; almost all cells die immediately, without dividing. This indicates a fundamental difference in the mode of action between x-rays and ultra-violet light. This difference applies not only to all other algal species tested but also to yeast. It is suggestive to think that the killing action of x-rays is more on a genetical level, that of ultra-violet light more plasmatic, *e. g.*, through protein denaturation or photo-oxidation of enzymes.

Radio-protection and photo-reactivation. — In order to gain knowledge about the mechanism behind the biological effects of radiations the radio-sensitivity of the cells has been studied under various conditions. The possibility of protecting the cells during irradiation has been the subject of special investigation. It is known that hypoxia counteracts the effects of ionizing radiations, and so do also certain chemicals, especially substances containing —SH groups.

The diagrams of Figs. 3 and 4 show that this is also true for these algae. The percentage of surviving cells is placed *versus* the x-ray dose. It is found that cystein (Fig. 3) and thiourea (Fig. 4) protect against x-rays as compared with irradiation in buffer controls; and this ability is also shared by other —SH compounds, *e. g.* glutathion, dimercapto-propanol and thioglycolic acid. That it need not necessarily be —SH compounds, however, is shown in Fig. 5. Here, another reducing substance, ascorbic acid, *i. e.* vitamin C, is also rather protective.

Hypoxia, obtained by means of bubbling nitrogen through the cultures during irradiation, also exerts a profound protection against



Figs. 1—2. Survival diagrams after irradiation with x-rays and ultra-violet light. — Figs. 3—5. Survival curves after x-irradiation in the presence and absence of protecting chemicals. Fig. 6. Probit lines showing chemical protection against ultra-violet light. — Fig. 7. The effect of visible light during x-irradiation. — Fig. 8. The effect of illumination with visible light after ultra-violet light. — Figs. 9—12. Growth curves for the normal type and a mutant requiring organic energy. — Figs. 1 and 2 refer to *Chl. eugametos*, all others to *Chl. Reinhardi*.

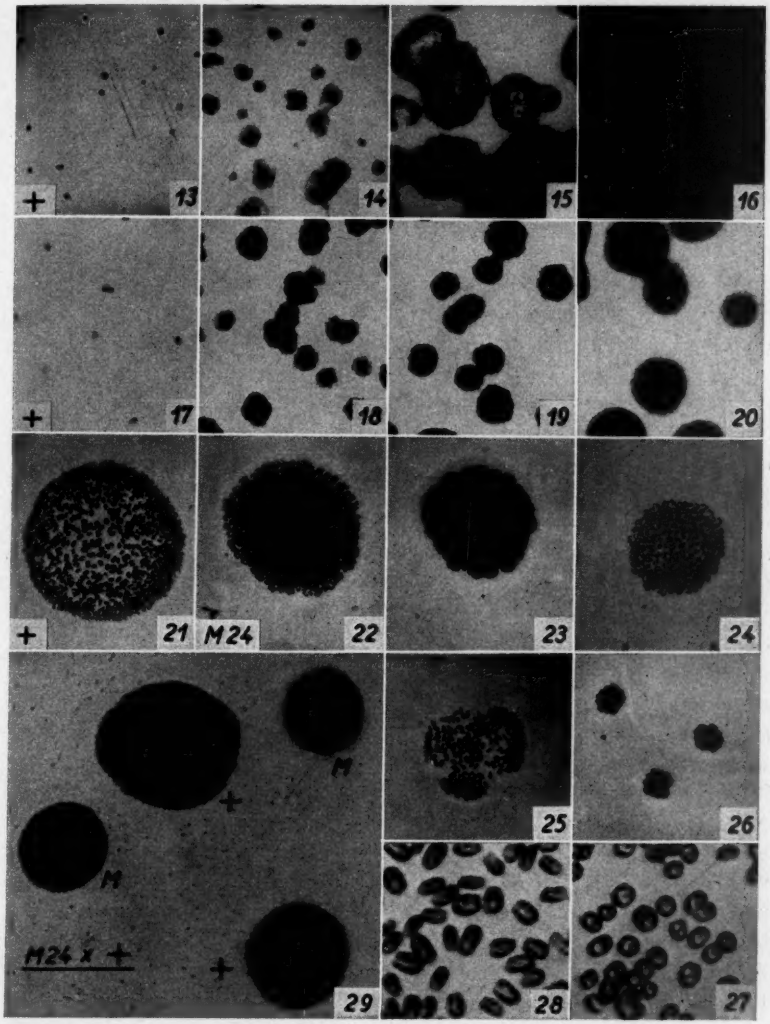
x-rays on these algae, but not against ultra-violet light, so far as preliminary experiments show.

The chemical radio-protection, however, applies not only to ionizing radiations as x-rays but also to ultra-violet light ($\lambda = 254$ m μ), as shown in Fig. 6. The survival fraction is here transformed into probit of survival, which is placed *versus* the dose. This figure, therefore, also demonstrates the application of probit analysis to these effects. A probit of 5 means 50 % survival. Some other, non-motile algae were used in these experiments, which had to be arranged in such a way that the relatively high ultra-violet absorption of the protecting compounds did not reduce the UV-dose received by the cells. This was achieved in the following way: A dilute suspension of the cells with or without the protecting chemical was filled into a blood-counting cell, covered with a quartz glass. This cell was then inverted. The algae were allowed to sediment down into the quartz glass and were then irradiated from below. In this way there was no or only 1—2 μ of fluid between the algae and the lamp. Protecting chemical was thioglycolic acid. Fig. 6 refers to a *Hormidium* species, but the protection also applied to the other non-motile algae tested, *Chlorella*, *Scenedesmus* and *Sticho-coccus*.

Thus we see that a reduction of the red-ox potential of the medium implies a radio-protection. But if this is so we might, *a priori*, expect that visible light would act in a sensitizing manner on these green algae, as photo-assimilation means a temporary rise in the red-ox potential of the cells. This is also found to be so, as shown in Fig. 7. Cells x-rayed in darkness show a much higher survival than cells x-rayed in light.

Photo-reactivation, *i. e.* the »rescuscitation» of irradiated cells with visible light, is enormously potent also in these organisms, as shown in the diagram of Fig. 8. The curves in this diagram, or rather the surfaces of it, show the increase in the percentage of surviving and end-point killed cells for one and the same ultra-violet dose, when the cells are illuminated with visible light (10.000 lux) for various times immediately after UV-irradiation. It is interesting to note that it is the zero-point killed cells which are primarily rescuscitated, *i. e.* those cells which have presumably been mostly killed through protoplasmic injury.

Mutations influencing drug resistance. — Experiments with induced mutations to streptomycin and sulfonamide resistance indicate that convenient, quantitative mutation tests may be worked out in these algae as in bacteria or in fungi. The photos of Figs. 13—16 are



Figs. 13—16. The growth on streptomycin-agar of the normal type (Fig. 13) and three mutants with increased streptomycin resistance. — Figs. 17—20. The growth on sulfonamide-agar of the normal type (Fig. 17) and three mutants with increased sulfonamide resistance. — Fig. 21. A colony of the normal type. — Figs. 22—26. Comparable colonies of some mutants (80 ×). — Fig. 27. A round-celled mutant. — Fig. 28. The normal type (400 ×). — Fig. 29. The four tetrad colonies after the cross M 24×the normal type. — All figures refer to *Chl. Reinhardi.*

taken from the same agar dish containing 20 micrograms of streptomycin per ml agar, and they show the colony size after one week of *Chlamydomonas Reinhardi* and three mutants with increased resistance to streptomycin. There is a great variation in sensitivity among the mutants. In Fig. 16 the colonies have quite grown together. Even towards sulfa-drugs it is easy to induce mutations with increased resistance, as shown in the analogous Figs. 17—20. These photos are taken from an agar dish containing 400 micrograms of sulfamethylpyrimidin per ml agar. This drug is the most active one of a series of sulfa-derivatives tested. In the case of increased resistance to sulfonamide the variation in sensitivity among the mutants is strikingly smaller. The increased resistance is specific to the drug in question. In no resistant mutant has there been any indications of a dependence on the drug, as they usually grow better in the absence of it. The growth on drug-free agar medium is somewhat reduced in some mutants but usually equals that of the normal type.

These algae seem to be perfectly resistant towards penicillin. *Chlamydomonas eugametos* already spontaneously possesses a very high degree of resistance to sulfonamides. Towards some of them it is quite resistant even in saturated solutions. Possibly this has something to do with the obligatory autotrofism of this species to be mentioned in the following section.

Physiological mutations. — Concerning the physiology of these algae it is interesting to note that *Chlamydomonas eugametos* is obligatorily autotrofic. which means that it is indifferent to organic nutrition, and consequently can only be cultivated in light. *Chl. Reinhardi* is more plastic and can be cultivated either autotrofically on pure mineral nutrition or heterotrofically in complete darkness on an organic energy source, *e. g.*, acetate or glucose. Both species can use inorganic nitrogen either as nitrate or as ammonia, but *Chl. eugametos* definitely prefers nitrate, whereas *Chl. Reinhardi* probably prefers ammonia. This difference may have some evolutionary connection with their different liability for mixotrophic growth. These nutritional differences together give quite a different basis for works with physiological mutations in these two species.

Hitherto physiological mutations have been produced only in *Chlamydomonas Reinhardi* with the same methods as those used in bacteria. An irradiated suspension is plated on a »complete medium», containing, *e. g.*, acetate, glycin, tryptos, malt extract, casamino acids, and yeast extract. The colonies formed on this agar are then trans-

ferred parallelly both to mineral medium and to new complete medium. Those clones which grow differently on the two kinds of medium are then isolated for further investigation.

The most common type of nutritional mutants obtained are those which grow either not at all or very slowly on mineral agar, but the growth of which is greatly promoted by addition of organic nutrition. Figs. 9—12 demonstrate such a case. They show the growth curves of the normal type and the mutant M 26 in mineral solution and in a solution with an addition of acetate. Growth is measured photometrically as extinction at 660 m μ. 4.000 lux means light saturation to the normal type, so that an increase in light intensity or an addition of acetate does not promote growth. The behaviour of the mutant is interesting. In mineral solution it does not show any measurable growth at all, but on acetate it grows quite well, especially at 10.000 lux. When grown in darkness it is not possible to distinguish this mutant from the normal type; they grow equally well. Its respiratory functions thus seem to be intact, but its ability to use light as the sole source of energy is impaired.

Another mutant, M 44, shows a rather peculiar behaviour. When grown on mineral medium it looks like the normal type. When grown on »complete medium», on the other hand, it grows for some days but then the colonies, comprising about some hundred cells, turn chlorotic and later quite white, and finally they die. This happens to almost all colonies except a few ones (perhaps 1 in 100 or 1.000), which attain a very dark-green colour and continue growing quite well into large, convex and dense colonies. Once adapted like this, the mutant grows quite well on »complete medium», but this adaptation disappears if subcultured on mineral medium. The factor in the medium causing this reaction is present in decreasing concentrations in casein hydrolysate, malt extract, yeast extract, and peptone. Several simple organic substances have failed to give the reaction.

Morphological mutations. — Morphological mutations and mutations with changed colony shape may also be induced. Fig. 21 shows a colony of the normal type, and Figs. 22—26 show comparable colonies of some mutants. Those in Figs. 22—24 belong to a very common type of mutations, which grow in thick, dense and convex colonies instead of the flat ones of the normal type. Figs. 25 and 26 show two slow-growing mutants. The mutant in Fig. 27, shown at higher magnification, has nearly round cells as compared with the more elongated ones of the normal type in Fig. 28.

Another mutant, M 29, has large cells, about twice the normal cell volume. Some mutants, M 62—M 66, were selected for looking more light-green. The lighter colour of these mutants was, however, due to a more disperse growth habit, leaving much space between the cells. True chlorophyll mutations with a reduced pigment content do not appear to be common. A few light-green types have been found, *e. g.*, M 1, M 3 and M 5. The growth of these chlorotic mutants is usually much reduced.

Genetical analysis. — As a material for mutation experiments these algae have the great advantage of being accessible for genetical analysis. Crossing experiments have so far been carried out only to a small extent, since still some technical difficulties have to be overcome, and further rationalizations of the methods have to be worked out.

Hitherto a few of the morphological mutants have been crossed with the normal type and they have all shown normal segregation.

Fig. 29 shows the colonies resulting from the four cells obtained at the zygote germination after a cross between the convex-growing mutant M 24 (*cf.* Fig. 22) and the normal type (*cf.* Fig. 21). Two of the tetrad colonies are convex-growing and two are of the normal type.

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